Jan Evangelista Purkyně University

Faculty of the Environment

Laboratory Training in Soil Science

Lenka Zoubková

Ústí nad Labem
2014

Title: Laboratory Training in Soil Science

Author: Ing. Lenka Zoubková

Scientific editor: Ing. Sylvie Kříženecká, Ph.D.

Reviewers: RNDr. Michal Řehoř, Ph.D.
Ing. Martin Dubský, Ph.D.

Language correct.: ELSEVIER

This publication was supported by the OP Education for Competitiveness project: EnviMod – Modernization of education in technical and natural sciences at UJEP with respect to environmental protection.
Reg. No.: CZ.1.07/2.2.00/28.0205

Free copy

ISBN 978-80-7414-800-2 (brož.)
ISBN 978-80-7414-801-9 (brož., angl. vyd.)
ISBN 978-80-7414-842-2 (online: pdf)
ISBN 978-80-7414-843-9 (online: pdf, angl. vyd.)

Content

Appendix list

Appendix I: Field record

Appendix II: Nomogram

Appendix III: Particle size curve

Introduction

This study material is called ‘Laboratory Training in Soil Science’, and it originated from the project OP Education for Competitiveness: EnviMod – Modernization of education in technical and natural sciences. This project was used at UJEP, and it addressed environmental protection and key activity KA 03, for the purpose of modernizing the current study material.

This textbook is intended for the second-year full-time students of the Environment Conservation study programme, who are required to pass laboratory training within the scope of the 1PED Soil Science and Protection subject. The text offers a comprehensive description of methods that are usually performed in certified laboratories; sampling descriptions and subsequent sample treatments are included.

Because basic information and pedologic analyses are involved, this study material was written in both Czech and English versions to meet the needs of both Czech and foreign students.

1 Soil field surveys

1.1 Terrain reconnaissance

Terrain reconnaissance occurs at the beginning of each soil field survey. Its primary aim is to provide more accurate data either during the preliminary literature survey or in the field itself. Alterations that are not included in a working map (e.g., new communications, water reservoirs, etc.) and data on the geological composition, underground water influence, growth conditions or anthropogenic impact must be recorded during the reconnaissance process.

The probe excavation locations, especially with respect to terrain relief, vegetation composition, geological, lithologic and hydrological characteristics, must be marked on the map after terrain reconnaissance. Several basic probe lines are set down to main relief forms (uphill and valley positions, upper, lower and middle parts of slopes with different orientations, plateaus etc.). It is very important to decide where the probing will be initiated. A place where consistent soil characteristics are expected (e.g., a plane surface) is usually recommended. The best method is to choose from spacious, high-altitude plane areas, where no runoff or impurity depositions are present, indicating that local soil conditions are undisturbed and outstanding. Afterwards, we continue along the slope to the foothill, where soil conditions tend to be more complicated.

This basic-sounding system is completed by the other probes used to characterize the soil cover of the service area fully and to maintain the standard density of the sounding network. Generally, the more heterogeneous the geomorphological, lithologic, hydrological and stand conditions, the denser the sounding net should be. The probes must be more or less equally distributed throughout the whole area of interest and every different soil site must be characterized by at least one probe.

1.2 Probe excavation

The excavation of soil probes can be performed manually, by shovel, spade or pickaxe. The front side of the probe is usually oriented opposite to the sun in a flat terrain; the front side is oriented opposite to the slope on steep land, meaning that the longitudinal

axis is perpendicular to the contour lines. Both the front and lateral sides of the probes must be vertical (perpendicular to the bottom).

The ground plan of the probe usually has a rectangular shape. The probe must be deep enough to decisively represent the soil strata, and large enough to find each individual horizon. The widths of the probes usually range from 60 to 80 cm, the longitude is approximately 150 – 200 cm, and the depth is 120 – 200 cm until reaching the parent rock, seat rock or groundwater level. The soil of the humus layer is separated from the soil of the bottom layer during the excavation process to be sure that the bottom layer will be recovered with the appropriate soil. It is customary that the soil from the bottom layer is shovelled not above the described probe side but on the other side.

Special soil cutters can be used to collect additional information. This method is not time consuming, but it provides only partial information about the whole soil profile, and thus, not all of the morphological characteristics can be measured. The other disadvantage is that the sample could be contaminated by soil from the top horizons and possibly yields inaccurate results.

1.3 Field recording and soil profile descriptions

A soil profile characterizes a vertical cut through the soil strata. Its description (i.e., stratigraphy and morphology) is reported in the field record (Appendix I). It is essential to record all of the necessary information about the given locality first.

Genetic horizons and their morphological characteristics are determined at the front wall of the probe. This wall must be cleansed by using a spade or shovel before describing the soil profile, to make its morphology and stratigraphy visible. A tape measure is then placed inside the probe to measure the thickness of particular horizons. When describing the soil profile, we always start with the upper horizon and gradually continue to the lowest one, which means the soil matrix, parent rock or groundwater level. All of the morphological characteristics of a given horizon must be recorded.

Among the major morphological characteristics are the following:

- diagnostic horizons and their thicknesses
- transition horizons and their characteristics
- colour
- structure
- texture and soil skeletons

- moisture
- consistence
- new formations
- carbonate and soluble salt contents
- porosity and cracks
- root distribution and biological activity

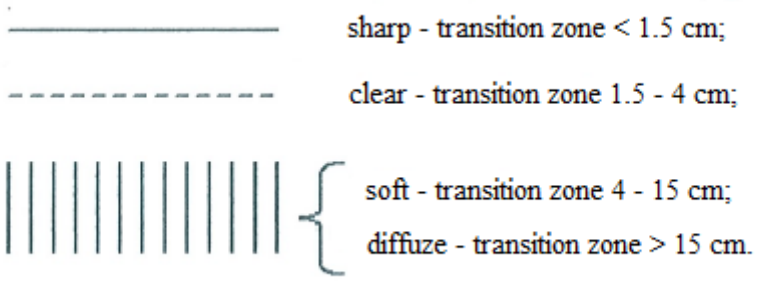
1.3.1 Diagnostic horizons and their thicknesses

Soil horizons are the layers in the soil profile that originate from soil-forming processes and carry typical properties and morphological characteristics. Their nomenclature, description and signature are mentioned in Buol et al. (2011), which could serve as a study material for foreign students.

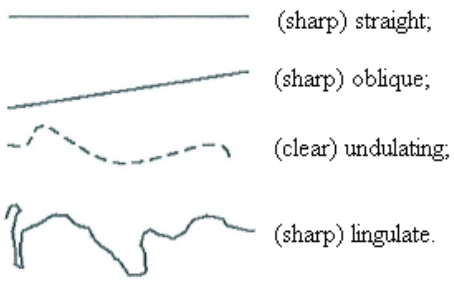
1.3.2 Transition horizons and their characteristics

The characteristics of transition horizons are represented by marks (among the indexes for the horizons) and expressed verbally (at the end of the description). A graphical illustration is shown below (Fig. 1).

Fig. 1: Transition horizons and their characteristics, a graphical illustration



In terms of the above-mentioned categories, the following transition is distinguished:



1.3.3 Soil colour

The soil colour is one of the most important features of the soil. It can be determined at the current soil moisture under field conditions; however, it is better to analyse samples of dry, eventually capillary moistened soil under laboratory conditions. The colour is usually influenced by the presence of soil particles, such as soil humus, substances made of iron, aluminium and manganese, calcium carbonate and various coloured minerals. It can be further influenced by soil moisture, oxidation and reduction processes and others.

Both subjective and objective classification methods are applied. Regarding the subjective ones, colour classification consists of two or three words, in which the first word stands for the intensity, the second for hue and the last marks the basic colour (e.g., dark grey-brown).

Munsell Soil Colour Charts belong to the standard methods of soil colour classification. They identify the colour according to three major variables, namely hue, value and chroma. The hue is a dominant spectral colour that is related to wavelengths of light. The primary dominant colours are red (R), yellow (Y), green (G), blue (B), purple (P) or their transitions. The capital letters in brackets correspond to the symbols used in the Munsell Charts. The value represents the total amount of reflected light and it marks the degree of soil

lightness or darkness. It indicates the level of similarity to white or the level of dissimilarity to a black colour. The chroma stands for the purity or strength of the spectral colour. These three variables are combined into reference charts, which cover the soil colour range. The various hues are arranged by page, with one hue to a page, in the Munsell Charts. The value units are arranged vertically and the chroma units are arranged horizontally on each page. The notation is interpreted for example as 10 YR 6/3, in which 10 YR stands for hue, 6 stands for value and 3 is the chroma.

Most soil horizons consist of intimately mixed colour patterns. The dominant colour of the horizon and the colour heterogeneity caused by gley processes (e.g., mottling) are described in terms of occurrence, quantity and contrast. The occurrence is given as a percent of differently coloured areas with regards to the hue and is divided into the following:

- small – differently coloured areas make up 10 % of the total
- middle – differently coloured areas make up 10 – 25 % of the total
- high – differently coloured areas make up more than 25 % of the total

The sizes of the differently coloured areas are determined in relation their diameters and are evaluated as follows:

- small – a diameter smaller than 5 mm
- middle – a diameter between 5 and 15 mm
- large – a diameter larger than 15 mm

The contrast in differently coloured areas is valued as follows:

- weak – differently coloured areas are of the same colour as the basic horizon colour
- obvious – differently coloured areas are evidently different from the basic horizon colour
- strong – differently coloured areas are markedly different from the basic horizon colour

1.3.4 Soil structure

The soil structure is a spatial distribution of fundamental soil particles and soil aggregates. Individual soil horizons can record the various conditions of soil material as follows:

- fused condition (fused structure) – soil particles are united into a continual mass that is not divided into different structural elements (soils with high clay particle contents)
- elementary condition – soil particles are not joined into aggregates; they could be freely placed side-by-side or united by clay coatings etc. (sandy soils)
- aggregate condition – one part of the soil material forms aggregates or records system of separation sheets that consequently cause disintegration to structural elements of a particular shape and size (peds)

Structure classifications are made according to the shape, edge formation and size as follows:

- Isometric structural elements (all three proportions are equally developed)
    - Sphere structure – indistinctly developed and freely stored edges and sheets, considerable inter-aggregate porosity, porous aggregates
        - cloddish – more than 50 mm
        - cloggy – between 50 and 10 mm
        - breadcrumbs – between 10 and 5 mm
        - slight breadcrumbs – between 5 and 1 mm
        - dusty – less than 1 mm
    - Grain structure – clearly developed and freely stored edges and sheets, considerable inter-aggregate porosity, partially porous aggregates
        - granular – between 10 and 5 mm
        - slightly granular – between 5 and 1 mm
    - Polyhedric structure – clearly developed and closely stored edges and sheets, low inter-aggregate porosity, partially porous aggregates
        - roughly polyhedric – more than 20 mm
        - polyhedric – between 20 and 10 mm
        - slightly polyhedric – less than 10 mm
- Anisometric structural elements (one or two proportions with different lengths)
    - Vertically elongated structural elements (prismatic structure) – a structure without a rounded upper part is called prismatic, and a structure with a rounded upper part is called columnar
        - roughly prismatic/columnar – more than 50 mm
        - prismatic/columnar – between 50 and 20 mm
        - slightly prismatic/columnar – less than 20 mm

- Horizontally elongated structural elements (platy structure)
  - roughly platy – more than 10 mm
  - platy – between 10 and 5 mm
  - laminar – between 5 and 2 mm
  - leafy – less than 2 mm

When describing the mixed characteristics of the structure by using two attributes, the last word indicates the dominant structure. Specific elements and structure alterations within the horizon are described freely.

1.3.5 Soil texture and soil skeleton

The soil texture and soil skeleton belong among the most important characteristics, which affect a variety of soil properties, e.g., moisture, thermal and air regimes and others. It is possible to determine these characteristics by visual and tactual tests directly under field conditions. The tactual test is performed by grinding moist soil between the thumb and forefinger, and the soil is thereby divided into three groups, namely clayey, loamy and sandy. Laboratory analyses are used for further details.

The soil texture is defined as the relative proportion of different soil separates represented in a soil material and their size category. The basic size fractions are mentioned in chapter 2.1, which is focused on laboratory methods for grain-size analysis.

Both the soil skeleton distribution in a soil profile and its relative degree of weathering belong to important indicators of lithologic substrate stratification. If the soil is skeletal, a gravel and stone survey is probed in addition to soil texture. Soil skeleton data are necessarily associated through the following characteristics:

- soil skeleton shape and perimeter – flat and angular fragments, rounded pebbles etc.
- soil skeleton hardness
- degree of rock fragment weathering
- soil skeleton deposition in a soil profile – parallel, flaggy, omnidirectional
- the petrographic composition of the soil skeleton – granite, gneiss etc.

1.3.6 Soil moisture

Soil moisture can be defined as the current amount of water in the soil. It changes during the year and depending on weather conditions, and it is represented by consistence, colour and other characteristics under field conditions. According to this indicator, soil can be distinguished as follows:

- parched – no signs of moisture; heavier soils form very hard, hardly crushed and hardened aggregates that do not crumble; lighter soils are loose and strongly dusty; and the colour of the parched soil horizon becomes considerably darker when wet
- dry – does not evoke a sense of cold; heavier soils are solid and more easily breakable, aggregates are hard to crush; lighter soils are easy to spill, they are broken when pressed with fingers; and the colour of the dry soil horizon becomes darker when wet
- mild – a flat hand is not moistened when pressing the soil but it evokes a sense of cold; heavier soils are breakable, they do not stick; lighter soils form small lumps when pressed with fingers but they cannot be formed; the colour of a mild soil horizon does not change when wet
- moist – a flat hand is moistened or the water distils when pressing the soil; heavier soils stick but do not become mushy; and lighter soils form conglomerates and various shapes when pressing with fingers
- wet – oversaturated by water, it becomes mushy and water drains away from the soil sample

1.3.7 Consistence

Soil consistence can be characterized by means of cohesion (the binding of soil particles together) and adhesion (the sticking of soil particles to different impurities). When the consistence is determined, it is necessary to distinguish the following:

- adhesiveness in wet conditions – the degree of adhesiveness is evaluated when the wet soil is pressed with the fingers (thumb and forefinger) and subsequently,
    - non-sticky soil – the soil does not remain on the fingers, it falls easily and completely

    - slightly sticky soil – some soil adheres to the fingers but it still easily and completely falls, and no resistance is felt when the fingers are taken away
    - sticky soil – soil adheres to both fingers, and a slight resistance is felt when the fingers are taken away
    - very sticky soil – soil adheres firmly to both fingers, and considerable resistance is felt when fingers are taken away
- plasticity under wet conditions, the soil is divided according to the plasticity index as follows:
    - non-plastic – soil cannot be formed into a roll
    - slightly plastic – soil can be formed into a thicker roll with difficulty
    - plastic – soil can be formed into a 1- to 3-mm-thick roll, and the rolls break down when curving
    - very plastic – soil can be formed into a roll with a thickness of less than 1 mm, and the rolls do not break down when curving
- resistance under moist/dry conditions as follows:
    - loose soil – cohesion-less soil material, dusty
    - friable soil – soil disintegrates after very slight thumb and forefinger pressure
    - cohesive soil – soil disintegrates after slight resistance between thumb and forefinger
    - (strongly, weakly) dense soil – a knife penetrates the soil under higher pressure
    - hard/rigid soil – soil disintegrates after strong pressure that is not caused by a thumb and forefinger, a knife penetrates 1 – 2 cm into the soil under higher pressure
    - very rigid/very hard soil – soil disintegrates after strong pressure that is not caused by fingers, a knife does not penetrate into the soil

1.3.8 New formations and concretions

New soil formations are defined as aggregates that originated during the soil forming process. They differ from the original soil especially because of their dissimilar colour and consistence. They can be divided according to their origin as follows:

- the following new formations originating from calcium carbonate ($CaCO_3$) translocation and accumulation:

    - pseudo-mycelium – white crystalline coatings of $CaCO_3$, in a similar shape to that of mushroom mycelia fibres, filling of soil tunnels and pores caused by roots or by edaphon
    - soft agglomerations of $CaCO_3$
    - nodules – rounded or elongated tough concretions
- the following new formations originating during the process of illimerization (clay and free sesquioxides translocation):
    - sprinkles (so-called powders) – in eluvial parts of the soil profile; accumulation of minerals (silica, sand and dusty fractions)
    - colloid coatings (films) – in illuvial parts of the soil profile; dark brown or dark grey-brown clay layers at the top of structural particles, fissures and pores; organic and mineral, different colours from the inner parts of aggregates
    - brown or red-brown stripes (in sand) – stripes with a thickness between 1 and 2 cm; originating from geologic or old pedologenetic processes
- the following new formations originating during the process of podzolization (free oxides translocation):
    - grains of sand/dust free from coatings in the eluvial horizon
    - rusty-brown coatings on sand grains in the illuvial horizon
    - undulating zones (an increased amount of free oxides must be present)
    - ortstein – strongly cemented new formation in illuvial horizon
- the following new formations originating from subsurface movements and volume changes:
    - slickensides – shiny sloping surfaces formed by high pressures during the repeated process of swelling and shrinking; typical for heavy soil
- the following new formations originating under the influence of groundwater and stagnant water:
    - the result of oxidation and accumulation
        - manganate coatings – black shiny coatings on the surface of structural elements in periodically moistened surface horizons
        - ferrous-manganate concretions – columnar red-brown or black-brown formations; found in periodically moistened surface horizons

        - rusty stains and coatings – red-yellow, red-brown or ochre-brown stains on the surface of structural elements; found in periodically moistened surface horizons
        - iron stain – cemented form of ferric hydroxides; origination from groundwater precipitation (G_{OR} or B_{OS} horizons)[1]
    - the result of reduction and depletion
        - reduced and depleted stains – irregular alternation or prevalence
        - mottling – the polygonal net formation of light green-grey depleted and reduced areas; fissures, surface of division planes and aggregates; fossil mottling is often typical for deluvium and loess loams
- the following new formations originating from biological activity:
    - mole chambers – tunnels made by larger animals; often filled by soil from other horizons
    - tunnels made by earthworms
    - cavities made by roots
    - humic stains

1.3.9 Carbonates and soluble salts

Carbonates in the soil are primarily represented by calcium carbonate ($CaCO_3$). Their determination is performed by bubbling the soil, which reacts with diluted HCl (10 %). The intensity and duration are measured. The evaluation is as follows:

- slight or noticeable bubbling – from 0,3 to 3 % $CaCO_3$; marked with (k) – e.g., Ac(k)
- intense and long-lasting bubbling – more than 3 % $CaCO_3$; marked with k – e.g., Ck

It is important to note if the carbonates are present in the whole soil material or only in the soil skeleton or concretions.

The presence and quality of soluble salts are determined by using both leachate (app. 1:5) and groundwater. The following ions can be subsequently determined in a small volume:

- Cl^- – by using $AgNO_3$ in acidic medium (2 or 3 drops of HNO_3); white coagulate occurs
- SO_4^{2-} – by using $BaCl_2$ in acidic medium (2 or 3 drops of HCl); white coagulate occurs

[1] Rem.: G_{OR} horizon = gley redox horizon
B_{OS} horizon = oxide ochrous horizon

- HCO_3^- – by using methyl red and methyl orange; a yellow colour results
- CO_3^{2-} – by using 1 or 2 drops of phenolphthalein when warming; yields a light violet colour

1.4 Collecting soil samples

Soil samples represent one part of the soil horizon that is collected to perform laboratory analyses immediately after the soil profile description. The sample must fulfil the following two major criteria in terms of the sampling itself:

- it must not be contaminated by other samples (e.g., by samples from the horizons above or by heterogeneous materials from the surrounding environment)
- it must be homogeneous and representative

Soil samples are taken from the front part of the probe from the lower part of the soil profile to the upper part to prevent contamination. In the case that the horizon is less than 5 cm thick, soil samples are taken from the entire horizon, in the case that the horizon is more than 30 cm thick, two samples are taken, namely an upper one and a lower one. The amount of sample depends on the aims of the pedological survey but it should not be less than 1 kg. With respect to skeletal soil, extra soil (2.5 kg) from the organic horizon is sampled to determine the skeletal portion.

The soil is sampled by using a shovel or a knife, and it is placed in sacks that were labelled in advance. The label should consist of the name of the sampling location, the sampling pit and sample numbers, the depth of the pit and the appropriate date. The removed and labelled soil samples should be stored in a dry and ventilated room. The duration for which they are closed inside the sacks should be as short as possible. In the laboratory, the samples are overspread so the layer is a maximum of 15 mm thick and is air-dried. Bigger nodules are simultaneously disintegrated. It is forbidden to dry the samples under the sun or other artificial heat sources. It is also necessary to protect the samples from contamination during the drying process.

When physical analyses are needed, undisturbed soil samples are used. These samples are removed by using steel rings, which are 5 cm high and have a volume of 100 cm^3 (for special instances, e.g., permeability determinations, rings of greater volume (1 000 cm^3) can be used). The soil is sampled in the opposite direction from disturbed samples, which means from the upper horizons to the lowest ones. Steel rings are sunk into the soil by gradual

pressure; it is very important not to pinch the soil. When the ring is full of soil, it is dug out and the emerging soil is cut with a knife. Afterwards, the physical ring is covered, fixed with a rubber band and put into the sack. Again, all of the important characteristic of the surrounding locality must be written down. If anything goes wrong with the undisturbed sample removal (e.g., a small part of the soil peels off or a stone is found in the ring), it is necessary to repeat the sampling. Undisturbed soil samples should be transported to the laboratory and used as quickly as possible.

If we need to determine the actual soil moisture only, desiccants are used for the sampling. These are aluminous dishes with a 50 – 100 cm^3 volume. Again, it is very important to use the samples as quickly as possible, although, in this case, the analysis can be performed 24 hours later or the desiccatives with soil samples can be stored in a room at a temperature less than 4 °C.

As far as the microbial analyses are concerned, germ-free sealing dishes for the undisturbed soil samples are used.

1.5 Sample preparation for laboratory analyses

Disturbed soil samples, which are destined for non-physical analyses, can have a mixed character, which is why homogenisation is necessary. The samples are mixed on a flat surface (e.g., a sheet of plastic foil), overspread in a square shape and cut by two diagonal lines (this process is called quartation). When the soil is quadrisected, the two opposite parts are removed and the rest is taken for the analyses.

Air-dried and homogenized samples are analysed in the following two forms:

- fine grained soil I – the diameter of the particles is less than 2 mm

  Noticeable particles of the soil skeleton, plant and animal residues are removed from the air-dried soil sample, and then the remaining sample is sorted through a sieve with a mesh diameter of 2 mm. Particles of soil skeleton cannot be ground. This form of soil is used to determine the soil reactions, cation and anion exchange capacity according to Mehlich, grain-size analyses, specific weight and physical clay amount and sorption complex of non-carbonate soils according to Kappen and others.
- fine grained soil II – the diameter of the particles is less than 0.25 mm

  To obtain fine grained soil II, fine grained soil I is used. The sample is again sorted through the sieve in the same fashion, with the exception of the mesh diameter

(0.25 mm). This soil is used to determine the percentual content of oxidizable carbon and the total nitrogen content.

2 Laboratory analyses

2.1 Particle-size analysis

As mentioned previously, the soil texture is one of the important indicators that influence many soil properties. There are various methods for particle-size analysis. One of the simplest ones is a sieving method. Although it refers to the original tactual determination, it still ranks among the standard methods for fine grained soil II and especially fine grained soil I determination. Its principle is to sort soil through a set of sieves.

The methods for particle-size analysis can be further divided into sedimentation and elutriation. The sedimentation methods are based on the different velocities of varied grain size downfall. They are expressed by Stokes´ formula (1).

$$v = \frac{2}{9} * \frac{r^2 (\rho_1 - \rho_2)\, g}{\eta} \text{ [m/s]} \qquad (1)$$

v = sedimentation velocity [m/s]
r = particle semi-diameter [m]
ρ_1 = specific weight of sedimentation particles [kg/m^3]
ρ_2 = specific weight of liquid [kg/m^3]
g = gravity acceleration 9.81 [m/s^2]
η = dynamic viscosity of liquid [Pa/s]
For water (t = 20 °C) η = 0.001004 Pa/s

If the sedimentation velocity is replaced by an h/T formula, a time (T) when a particle of given diameter (r) reaches sedimentation trajectory (h) can be calculated (2, 3).

$$T = \frac{9}{2} * \frac{h\,\eta}{r^2 g (\rho_1 - \rho_2)} \text{ [s]} \qquad (2)$$

T = sedimentation time [s]
h = sedimentation trajectory [m]

$$r = \sqrt{\frac{9}{2} * \frac{h\,\eta}{T\, g (\rho_1 - \rho_2)}} \text{ [m]} \qquad (3)$$

The principle underlying elutriation methods lies in the particle's resistivity to the various velocities of the water current. They are described by Schöne´s formula (4).

$$d = 0.0314 * \sqrt[11]{v^7} \text{ [mm]} \quad (4)$$

d = diameter of drifted particles [mm]

v = velocity of ascending current [mm/s]

For velocity ranging from 0,1 – 12 mm/s

2.1.1 Grain size analysis

2.1.1.1 Sieving method

The sieving method is one of the oldest and easiest methods for particle size determination. It belongs to the separation techniques and its principle lies in the use of a set of sieves with different mesh diameters. The diameter decreases with the direction of the gravity transport for the analysed material.

A grain size analysis can be performed under dry or wet conditions. The result of the individual fraction content is given in percentages by weight. The grain size analysis also provides information on the figures with defined particle sizes, which indicates the primary advantage of this method. However, the impossibility of a direct density assessment in the suspension or in the current of air is a disadvantage, in addition to its time consuming and destructive character.

Laboratory equipment and reagents: analytical balance, set of sieves, sharp brush, beaker

Work process:

Approximately 1 kg of air-dried soil sample is placed on the set of sieves with gradually decreasing mesh diameters (10 – 0.1 mm) and passed through with the help of a sharp brush. The fraction remaining on the sieve of a particular mesh diameter is put into a beaker, which is weighed in advance, and its weight is determined. The percentual distribution of each fraction is then calculated from the total weight.

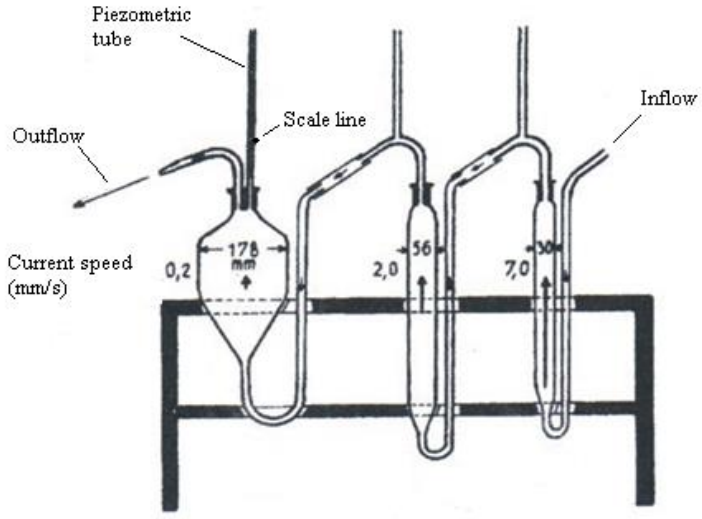
2.1.1.2 Elutriation method

The primary principle underlying elutriation method is indicated by the Kopecký flume apparatus (Fig. 2) utilization, where water flows among separate pipes in ascending direction and counteracts the sedimentation velocity of soil particles.

The apparatus consists of three glass cylinders of given diameters (30, 56 and 178 mm). The IV. category remains in the narrowest cylinder, where 50 g of soil sample is inserted. The III. category remains in the middle cylinder (56 mm) and the II. category rests in the largest one. The finest I. category runs away and is counted as the difference up to 100 %. When the elutriation process is over, given categories are washed out from cylinders and dried and weighed after cooling.

The elutriation method is not accurate enough because hard tap water of variable temperatures is used and all losses are at the level of the I. category, which is crucial for the soil class determination.

Fig. 2: Kopecký flume apparatus

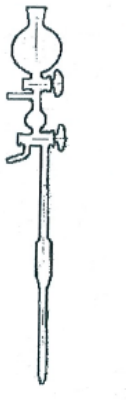


2.1.1.3 Pipette method

The pipette method belongs to the non-repetitive sedimentation methods. A soil suspension is stirred in a graduated cylinder and sample volumes from 20 – 25 ml are taken by a pipette from set depths and time intervals. Afterwards, the samples are dried and the percentual distributions of individual fractions are determined.

Laboratory equipment and reagents: pipette apparatus (Fig. 3), analytical balance, beaker (200 ml), watch-glass, laboratory oven, desiccator, dryer, sieve of mesh with a diameter of 0.25 mm, graduated cylinder (1 000 ml), sedimentation cylinder (1 000 ml), glass rod, timer, dispersing agent (sodium hexametaphosphate), distilled water.

Fig. 3: Pipette apparatus



Work process:

The charge of fine grained soil differs according to the soil class (10 g of heavy soils, 30 g of light soils). Place a weighed soil sample into a beaker and add dispersing agent and distilled water (the same amount of dispersing agent in ml as the amount of soil sample in g). Overlay the beaker with a watch-glass and let it stand for 24 hours.

After the elapsed time, boil the soil suspension for 1 hour, and replace the lost water with hot water. Transfer the cooled suspension into a graduated cylinder through a sieve and fill it with distilled water until the scale-line (1 000 ml) is reached. The fraction remaining on the sieve is placed into a laboratory oven in a dryer for 1 hour at 105 °C, and then it is cooled down in a desiccator and weighed (value D). Simultaneously, the dry matter is determined (weigh the same amount of fine grained soil as for the analysis itself and let it dry for 3 hours at 105 °C; after cooling down in a desiccator, weigh the sample and calculate the amount of dry matter).

Stir the tempered soil suspension for 1 minute with a glass rod in a sedimentation cylinder. Start a timer and remove the samples from three different depths during given time periods by using a pipette (Tab. 1). When the sedimentation period of individual fractions is reached, take 25 ml of soil suspension in a pipette. The time differs according to the depth as follows: 25 cm depth – 10 s, 10 cm depth – 12 s and 7 cm depth – 15 seconds before the sedimentation period is reached. It is necessary to regulate the speed at which the soil suspension is taken up; half the volume of a pipette must be filled during the sedimentation time.

Release the suspension from the pipette out into a weighed dryer and dry it in a laboratory oven (1 hour, 105 °C). Allow it to cool down in a desiccator and weigh it

(values of individual samples A_1, A_2 and A_3). A blank test is simultaneously carried out, and an identical amount of dispersing agent is put into a sedimentation cylinder, which is filled with distilled water (1 000 ml). The volume, which corresponds to the pipette apparatus, is taken (value V), and an evaporation value is determined (value C). In the end, a calculation for individual particle-size categories is performed (see formulas 5 – 8).

Tab. 1: Intervals for taking a soil suspension according to a soil fraction

Fraction (mm)	< 0.05	< 0.01	< 0.001
Sink (cm)	25	10	7
Suction (s)	20	25	30

$$\text{IV. category (\%)} = \frac{D}{\text{dry matter}} * 100 \quad (5)$$

$$\text{III. category (\%)} = \frac{(A_1 - C)(1\,000/V)}{\text{dry matter}} * 100 \quad (6)$$

$$\text{II. category (\%)} = \frac{(A_2 - C)(1\,000/V)}{\text{dry matter}} * 100 \quad (7)$$

$$\text{I. category (\%)} = \frac{(A_3 - C)(1\,000/V)}{\text{dry matter}} * 100 \quad (8)$$

2.1.1.4 Areometric method (according to A. Cassagrande)

The areometric method belongs among the densimetric methods based on the use of special densimeters (areometers) that monitor changes in the density of a soil suspension in water and in various soil particle diameters in terms of the sedimentation time changes. Density changes are caused by the sedimentation process; the density of the hydro-suspension falls with the increasing length of measurement.

This method is one of the non-repeated sedimentation methods, which means that all of the measurements must be performed within one sedimentation process.

Laboratory equipment and reagents: analytical balance, porcelain dish, laboratory spoon, timer, graduated cylinder (1 000 ml), glass rod, areometer, dispersing agent (sodium hexamethaphosphate), distilled water.

Work process:

The charge of fine grained soil I differs according to the amount of clay particles in the soil. In the case of clayey soils, the charge ranges from 20 to 30 g (in clays it is 10 g), as far as sandy-clay, loamy and clay-loamy soils are concerned, the charge is approximately 30 – 50 g and in case of sandy soils it is 100 g. The soil is weighed, placed in a porcelain dish and mixed with distilled water (200 ml). Dispersing agent is then added (10 ml of agent for every 10 g of fine grained soil I) and it is boiled for 1 hour. Occasional stirring is necessary to prevent the soil from burning on to the walls of the dish. The suspension is mulled at the bottom of the dish after cooling while the muddy part is poured directly into a graduated cylinder (1 000 ml). The suspension in the graduated cylinder is mixed by a glass rod for one minute before the measurement starts. When the glass rod is taken out, an areometer is put in its place and the first density measurement starts after 30 seconds. It is important to prevent the areometer from adhering to the wall of the graduated cylinder. The other measurements continue according to the following table (Tab. 2). After the fourth measurement is performed, the areometer must be taken out, washed with distilled water and can be put back into the graduated cylinder for one minute before the next measurement. This process is used for the other measurements as well.

Tab. 2: Recording the measured values

Locality:	Specific weight:
Sample No.:	Date of analysis:
Charge:	

Sedimentation time	**Measurement time (h:min:s)**	**Density reading**	**Temperature (°C)**	**Temperature correction**	**Grains diameter (mm)**	**SUM (%)**
30´´						
1´						
2´						
5´						
15´						
45´						
120´						
300´						

The results are analysed with a nomogram (Appendix II), in which the size of settling particles is determined depending on the individual sedimentation time.

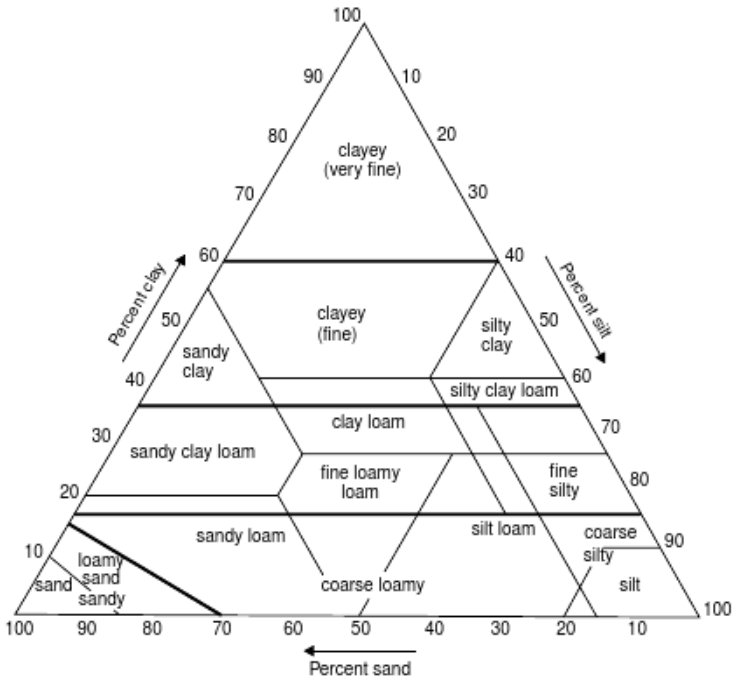
The last two columns of the table (Tab. 2) are used for particle size curve construction (Appendix III), which enables a percentual determination of individual fractions.

The soil type is identified according to the given values. A classification system according to Novák (Spirhanzl, 1954) is used in the Czech Republic. It is applied to particles with diameters below 0,01 mm (Tab. 3). A triangular textural diagram can be used as well (Fig. 4).

Tab. 3: Soil texture classifications according to Novák

Particle content < 0,01 mm (%)	**Soil type nomenclature**	**Basic soil type**
0	Sand	Light soil
0 – 10	Sandy	
10 – 20	Loamy sand	
20 – 30	Sandy loam	Medium heavy soil
30 – 45	Loamy	
45 – 60	Clayey loam	Heavy soil
60 – 75	Clayey	
> 75	Clay	

Fig. 4: Triangular textural diagram U.S.D.A.



(Sarkar & Haldar, 2005)

2.1.2 Soil skeleton analysis

The soil skeleton consists of soil particles with diameters greater than 2 mm. Its analysis is performed when a soil sample contains more than 10 % of these particles. The results can be expressed as the percentage by weight and by volume.

Determining the soil skeleton as the percentage by weight

An air-dried soil skeleton (approximately 500 g) is sieved through a set of sieves and individual fractions are weighed and expressed as percentages by weight. Sieving can be performed by a sharp brush, in this case a set of sieves with a mesh diameter from 2 – 30 mm is used, or with a water current, and a set of sieves with a mesh diameter of 2 mm is used. In the second case, the skeleton remaining on the sieve must dry in a laboratory oven (105 °C), cool down in a desiccator and be weighed.

A certain alteration in the results is possible and is usually caused by differences in the specific weight of the skeleton and fine-grained soil.

Determining the soil skeleton as a percentage by volume

The primary principle is to scale the volume of fractions gained from the determination as a percentage by weight. A particular fraction is placed into the graduated cylinder, poured over with a given amount of distilled water and when all of the air is removed, the skeleton volume is read and individual fractions are calculated in the percentage by volume. All of the volume determinations are must be performed at laboratory temperature (approximately 20 °C).

In contrast to the soil skeleton determination as the percentage by weight, determining the percentage by volume eliminates the difference in the specific weight of the skeleton and fine-grained soil.

The skeleton can be divided into the following three categories:

- coarse sand (2 – 4 mm)
- gravel (4 – 30 mm)
- stone (> 30 mm)

The coarse sand is taken into account only in samples in which it creates more than 50 % of the total. The soil is then called coarse-grained sand. Particles larger than 4 mm are described below (Tab. 4).

Tab. 4: Soil skeleton determination

Gravel and stone proportion (%)	Signifies
5 – 10	Gravel/stone infusion
10 – 25	Slight gravelly/stony
25 – 50	Medium gravelly/stony
50 – 75	High gravelly/stony
75 – 100	Gravelly/stony

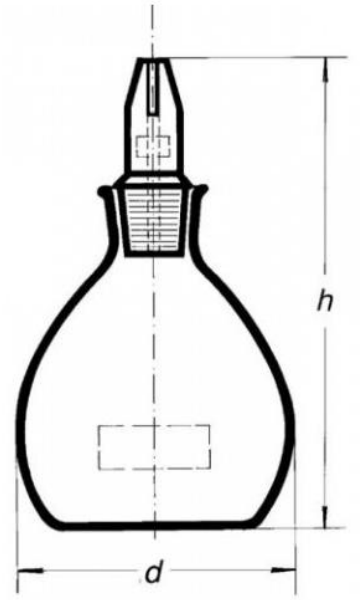
2.1.3 Specific weight determinations

The specific weight of a soil is defined as the weight of 1 cm^3 of soil sample that is completely free of gas and liquid phases. In terms of the specific weight, the soil porosity percentage is determined. The specific weight is one of the basic soil physical properties,

the values of which rise with increasing elements of high atomic weight and fall with the water ratio in a given rock mineral.

The pycnometric evaluation of a specific weight will be used for laboratory training purposes. The pycnometer (Fig. 5) is an accurately calibrated thin-walled glass bottle with a narrow mouth and a ground plug with its capillary leak. Its size is proportional to the volume of analysed sample. In the case of analysis itself, the pycnometer is filled to one quarter of its height with a soil sample.

Fig. 5: Pycnometer



(http://www.verkon.cz)

(http://www.helago-cz.cz)

Laboratory equipment and reagents: pycnometer, laboratory oven, desiccator, analytical balance, laboratory spoon, beaker, distilled water

Work process:

Weigh a clean, numbered pycnometer (value A) and fill it with distilled water to the brim. Insert the plug so the water passes through the capillary leak. Weigh the pycnometer again (value B). Pour out the water and put the pycnometer into a preheated laboratory oven (105 °C) for 10 minutes. After the elapsed time, let the pycnometer cool down in a desiccator.

Weigh 10 g of air-dried fine-grained sample I, put it in the pycnometer and weigh it again (value C). Then, fill half the pycnometer with distilled water and start to heat it up in a water bath. The distilled water in the beaker is simultaneously heated to eliminate CO_2.

During the desiccation process, the whole liquid phase was removed from the soil sample, which means that the pycnometer content consists of the solid phase only. The pycnometer is allowed to cool down to laboratory temperature and is filled with prepared, de-aerated distilled water. The plug is inserted again, and the whole thing is weighed (value D). Finally, the specific weight is determined according to simple calculation (9).

$$\rho_z = \frac{C-A}{B+(C-A)-D} \quad [g/cm^3] \tag{9}$$

2.2 Analysing undisturbed soil samples

The undisturbed soil sample is used for physical soil analyses. As mentioned earlier, an undisturbed soil sample is taken in the form of physical steel ring (Kopecký ring). For undisturbed soil samples, the actual moisture, absorptivity, maximum soil water capacity, the maximum soil water capacity according to Novák and total porosity can be determined during one work cycle.

Laboratory equipment and reagents: physical sampling ring (100 cm^3), analytical balance, watch-glass, filter paper, laboratory oven, desiccator, distilled water

Work process:

The numbered sampling ring must be weighed before the sampling (value V). After sampling, take the soil to the laboratory as fast as possible and weigh it without the top (value A). The ring should lay on a watch-glass when weighing. Value A represents real soil moisture. The ring is further saturated by distilled water through capillary action by using filter paper. Cover the upper side of the ring with a watch-glass to prevent evaporation. Saturate the soil minimally for 12 hours until the upper base is completely damp, which is indicated by glistening.

Remove the physical ring when saturated and let the redundant water drain away. Put the ring on the watch-glass and weigh it again (value B). This value indicates absorptivity and the sample is known to be capillary saturated.

Place the sampling ring on 4x folded filter paper and cover the top with a watch-glass. At this time, water will start to drain from the sample. Allow it to drain for 30 minutes and weigh the ring (value C). Put the ring on 4x folded dry filter paper again, cover the top

with a watch-glass and let it drain for 90 minutes. Weigh it (value D) so the result provides the maximum water capacity according to Novák (θ_{MKK}). Repeat the whole procedure for the next 22 hours. The retention water capacity (θ_{RVK24}) can be determined after weighing the soil sample (value E).

Place the ring with soil and weighed watch-glass (value S) into the laboratory oven (105 °C) for 24 hours (till the laboratory temperature is reached). Let it cool down in a desiccator and weigh it again (value F). This value provides a dry matter content determination (value H).

Particular soil characteristics can be calculated according to the formulas in table (Tab. 5a).

Tab. 5a: Evaluating specific soil physical characteristics I

Specific characteristic	**Sign**	**Formula**	**Unit**
Actual moisture	θ_{mom}	$\theta_{mom} = A - F$	% obj.
Absorptivity	θ_{ns}	$\theta_{ns} = B - F$	% obj.
Moisture 30-minutes	θ_{30}	$\theta_{30} = C - F$	% obj.
Maximum soil water capacity	θ_{MKK}	$\theta_{MKK} = D - F$	% obj.
Retention water capacity	θ_{RVK}	$\theta_{RVK} = E - F$	% obj.
Dry matter	H	$H = F - (V + S)$	g
Bulk density	ρ_d	$\rho_d = H/100$	g/cm^3
Capillary porosity	P_k	$P_k = \theta_{RVK}$	% obj.

When specific weight values are used (see Chapter 2.1.3), other soil physical characteristics can be determined (Tab. 5b).

Tab. 5b: Evaluating specific soil physical characteristics II

Specific characteristic	**Sign**	**Formula**	**Unit**
Total porosity	P	$P = (\rho_z - \rho_d) * 100 / \rho_z$	% obj.
Non-capillary porosity	P_n	$P_n = P - \theta_{30}$	% obj.
Semi-capillary porosity	P_s	$P_s = \theta_{30} - \theta_{RVK}$	% obj.
Aeration	V_z	$V_z = P - \theta_{mom}$	% obj.
Maximum soil aerial capacity	K_{MKKvz}	$K_{MKKvz} = P - \theta_{MKK}$	% obj.
Retention aerial capacity	K_{RVKvz}	$K_{RVKvz} = P - \theta_{RVK}$	% obj.

2.3 Soil moisture

The amount of water in the soil is determined as the soil moisture. The soil moisture determination methods are divided into direct, when the soil water content is measured, and indirect, when variables depending on the soil water content are measured.

One of the most used methods of soil moisture determination is the gravimetric method. Its biggest advantage is that the amount of water removed from the soil by the drying process is directly determined. The principle underlying this method lies in the destruction of the bonds between the water molecules and solid phase particles during the drying process. According to the international standard (ISO 11 465, 1993), the drying temperature should be 105 °C. When the volume of the sample is 100 cm^3, 24 hours of drying is enough. With a lower weight (e.g., 10 – 20 g), six hours are sufficient. Sometimes a small amount of hydroxyls can be released, which is why de-aerated places containing siccative, e.g., $CaCl_2$, P_2O_5 or concentrated H_2SO_4, are used when accuracy is required. In peat soils, lower temperatures (50 – 60 °C) are recommended to prevent the combustion of organic substances and consequent alteration in results.

The main disadvantage of the gravimetric methods is a destructive character. The soil profile and hydraulic properties are affected, and therefore the method is not suitable for long-term observation. In addition, this method is time consuming and the sampling is quite difficult.

For indirect methods of soil moisture determination, neutron probes, capacitance, gamma radiation, TDR (time domain reflectometry), electrical resistance and remote sensing methods are used, but we will not focus on them.

The results of a soil moisture determination are usually expressed as an index number less than 1 or as a percentage by mass and by volume.

2.3.1 Field capacity

The field capacity describes the amount of water that can possibly be held by soil for a longer period after saturation. It can also be described as a water supply indicator for plants.

Laboratory equipment and reagents: sampling probe, PVC sheet, wooden/steel board for fencing the area

Work process:

An area (2 x 2 m) is chosen and fenced by a wooden or steel board. Near this area of interest, individual soil samples are taken from different soil horizons by using a sampling probe to determine the soil moisture, porosity and bulk density. The amount of water that is added to the soil must be proportional to the volume of completely full pores, multiplied by 1.5. When water soaks into the soil, cover the soil with a PVC sheet and let it stand for 1 day (sandy soils) or 5 – 7 days (clayey soils). After the prescribed time, uncover the soil and take three soil samples from different parts of the area to determine the soil moisture. Repeat this procedure after 2 days, and when the moisture changes less than 1.5 %, we can consider it the result to be the field capacity (θ_{PK}).

The field capacity is strongly dependent on the grain-size distribution; the higher differentiation, the higher the θ_{PK} values. Various methods for field capacity determination are available and their results can differ slightly, but according to several authors, we can assess the values for specific soil classes (Tab. 6). The value for the soil moisture potential (pF)[2] in terms of the field capacity ranges between 2 and 3.

Tab. 6: The field capacity of specific soil classes

Soil class	Field capacity (%)
Coarse sand	9 – 14
Fine sand	15 – 20
Sand clay	20 – 25
Clay loam	25 – 30
Clay	30 – 40
Organic horizons	> 41

The optimum conditions for sowing provide sandy clay and loamy soils with an amount of sand particles of up to 15 %, with 20 – 30 % volumetric water capacity and a volumetric aerial capacity from 15 – 20 %. To evaluate the available water supply for plants, the field (full) water capacity (FWC), aerial capacity (AeC) and wilting point (WP) are used. The FWC corresponds to the amount of water that can be held by soil for a longer period. All capillary pores are filled with water; other pores are filled with air (AeC).

[2] pF values represent the energy of water fixation; pF = log |H|, where H stands for head pressure [cm] (e.g. pF 2 → 100 cm)

The FWC is determined by using a sand tank at a -10 kPa potential (100 cm under the pressure of a water column).

2.3.2 Wilting point

The wilting point indicates the soil moisture when plants are insufficiently provided with water and they start to fade. This measurement can be used as a lower bound for soil water that is available to vegetation. If good soil aeration is present, the wilting point should reach the highest values. An average wilting point was established for agricultural plants with respect to the fact that wilting is influenced by many factors (meteorological conditions, the developmental stage of plants, etc.). This average value reaches pF = 4.18.

The soil suction pressure increases with decreasing soil moisture. After the wilting point is reached, plant roots are able to drain almost no water from the soil. Wilting proceeds within a wide moisture range, which is why the limit point corresponds to the lower limit of this interval (with soil moisture determined along a -1 500 kPa potential). To determine the water content along -1 500 kPa, a potential pressure chamber is used. This amount of water indicates that water is unavailable to plants.

The amount of water between the FWC and WP is the available water supply (available water capacity AWC), an important indicator of soil drying.

The wilting point can be determined according to Váša (1959) when a disturbed soil sample is used.

Laboratory equipment and reagents: glass tube (diameter 12 mm, length 10 cm), burette, cotton-wool, drier cup, laboratory oven, spattle, marker, distilled water

Work process:

Close one side of a glass tube, allow it to stand in a vertical position and spill air-dried fine-grained soil at approximately 1 cm below the edge. Continuously add 1 ml of water from a burette so that a continual layer of water is not made. Allow the tube to stand in a vertical position for 24 hours to equalize the moisture. Mark a soil column at 2 cm high, as created by a border of wetness. Place this column in a dryer cup and determine the soil moisture (according to above mentioned procedure). The result will be expressed as a percentage by weight because we worked with a disturbed soil sample.

The wilting point value can be measured on the basis of I. the textural category distribution as a percentage by volume (10).

$$\theta_{BV} = 0{,}3 * \% \text{ I. category} + 4 \quad (10)$$

2.3.3 Determining retention curves

Retention curves describe a relation between the moisture potential (suction pressure) and soil moisture. Their progression depends on the textural and mineralogical composition, humus content, soil structure, bulk density and exchangeable cations. They are usually drawn into a graph at a semi-logarithmic scale because of the wide range of soil water potentials, which is expressed in a logarithmic scale. Retention curves are usually presented as pF values from a graphical point of view.

Retention curves and suction pressure can be determined under both field and laboratory conditions.

2.3.3.1 Laboratory methods of determination

The laboratory methods of determination can be divided into hypotonic and hypertensive methods. Soil is usually placed on a semi-permeable membrane (water permeable and air impermeable until a certain overpressure/underpressure value is reached) and it is exposed to suction pressure, which can be caused by air overpressure above the membrane or by water underpressure below the membrane. The membrane sucks water from the soil or gives water to the soil until equilibrium between soil moisture and applied pressure is established. Moisture is further set on the basis of weight (the weighing of the sample) or volume (measuring the amount of water).

The measurement is performed with undisturbed soil samples although there are several cases in which a disturbed sample can be used as well. With regards to pressures higher than 300 kPa, the results do not differ. The sample height should be up to 2 cm, especially because of the time duration (a considerable time is needed to reach the equilibrium). Because a draining part of the curve is usually being measured, the samples must be saturated in advance with boiled distilled water. Boiled distilled water mixed with toluene (ratio 1 000:1) is used for long-term measurements to restrict microbial activity. To reduce warming in warm-off the soil, a 0.01 mol/dm^3 solution of $CaSO_4$ is used.

Hypotonic apparatus utilization

Hypotonic equipment is used for pressures under 30 kPa (300 cm, pF 2.47), and those with suction-pumps make it up to 80 kPa (800 cm, pF 2.90).

Among the most common apparatus is the sand box (Fig. 6), which is used for pFs from 0 – 2.0. This equipment consists of a sand box with a control panel, suction levelling stand, water supply bottle with stand and a filter cloth (140-150 micron). It is necessary to use 50 kg of sand (grain size approximately 73 microns). Steel rings with soil are put into the box, and their number cannot be higher than 40.

A sand-kaolin box (Fig. 7) is used for pF 2.0 – 2.7 determinations. It consists of a sand-kaolin box with a control panel, suction levelling stand, water supply bottle, a vacuum pump with an automatic suction level control system, containers with sand (50 kg), a container with kaolin clay (2.5 kg) and filter cloth (140-150 micron). The retention curve determination is similar to that of the previous apparatus, and even the number of steel rings corresponds.

Fig. 6: Sand box

(http://www.ekotechnika.cz)

Fig. 7: Sand-kaolin box

(http://www.ekotechnika.cz)

Hypertensive apparatus utilization

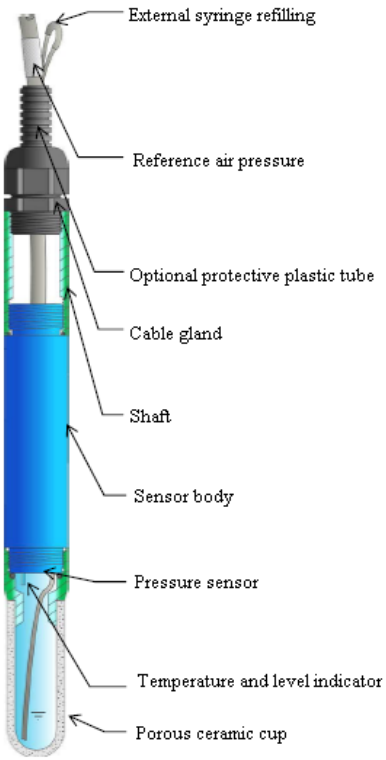
Hypertensive equipment is usually used for higher pressures (10^4 – 1.5 . 10^6 Pa). They are based on a hypertensive chamber with a semi-permeable membrane. Overpressure is

provided by a compressor unit or by a pressure tank with compressed air. A manometer is used for regulation.

2.3.3.2 Field methods of determination

Field methods of determination are performed by tensiometer (Fig. 8). The most important part of the tensiometer is its porous ceramic cup, the walls of which enable a hydraulic connection between the manometer and soil water.

Fig. 8: Tensiometer (type T8)

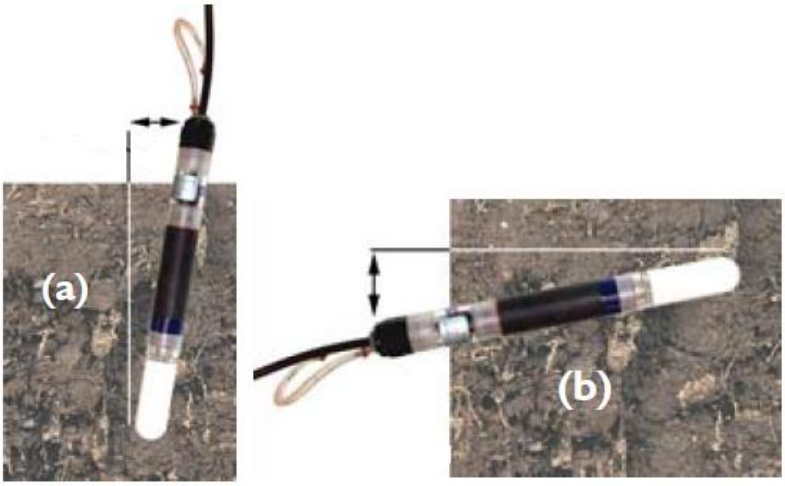


(http://www.ekotechnika.cz)

A standard tensiometer consists of a thick-walled plexiglass tube with lengths ranging between 15 and 150 cm. At the bottom there is a porous ceramic cup with a semi-permeable membrane, and at the top, it is possible to close a slot to which the manometer is connected. The inner part of the tensiometer is created by a shaft that is filled with de-aerated distilled water. If the soil is not saturated and water flows from the tensiometer, an underpressure, as recorded by manometer, is formed. However, if the soil is saturated enough there is no underpressure and the manometer shows a zero value.

A tensiometer can be installed in the soil in two ways. First is the downward installation, in which the position of the cup is lower than the end of the shaft (case (a), Fig. 9). Second is the upward installation, in which the position of the cup is higher than the end of the shaft (case (b), Fig. 9).

Fig. 9: Tensiometer installation



(Source: http://www.ekotechnika.cz)

Data-loggers or PC connections are used to read the measured values. The older types of tensiometers required manual reading; the increase in the mercury (mm) was multiplied by 12.5. The results were given in cm of water column. A special scale (1 cm/12.5) exists for easier reading, and a negative pressure high (value H) is reached. When the soil surface stands for the beginning of the "z" axes, the total potential can be expressed as follows (11):

$$\Phi = H - z \tag{11}$$

Interpreting results:

0 kPa:

Soil is normally fully saturated after a long-lasting rain or excessive irrigation. If this value is maintained for longer, it is necessary to improve the drainage system (roots suffer from a lack of oxygen and decay).

0 – 10 kPa (pF 0 – 2.01):

Soil holds surplus water, which is not necessary for plant growth. If water does not flow away in a few days, its presence is unfavourable and the drainage system must be improved.

10 – 20 kPa (pF 2.01 – 2.31):

The soil water capacity is reached, which means that the soil holds the maximum water needed for prosperous plant growth. When this value is reached, irrigation must be stopped because a higher amount of water would not be used by plants immediately. In the case of sandy soil and water-sensitive plants (e.g., potatoes), irrigation can be required within the pressure range from 15 – 20 kPa.

20 – 40 kPa (pF 2.31 – 2.61):

Soil water and soil air have favourable relations. With heavy and medium-heavy soils, irrigation is not needed. In the case of coarse sandy soils, irrigation starts when the pressure ranges between 20 and 30 kPa, and for light soil between 30 and 40 kPa.

40 – 60 kPa (pF 2.61 – 2.79):

Soil water and soil air are favourably related for heavy clay soils. Irrigation starts for medium heavy soils. Generally speaking, lighter soil means lower readings for irrigation.

60 – 80 kPa (pF 2.79 – 2.91):

Irrigation starts when the pressure ranges from 70 – 82 kPa in the case of heavy soils. The risk of plant death is higher in medium-heavy soils. Plants in sandy soils are damaged.

2.4 Soil reactions

The soil reaction is a basic physical-chemical soil property. It is given by the ratio between the concentrations of H^+ and OH^- ions in a soil solution. This ratio is expressed by a pH value. The roil reaction affects basic biochemical soil processes and nutrient uptake by autotrophic organisms.

Three basic forms of soil reaction are distinguished as follows:

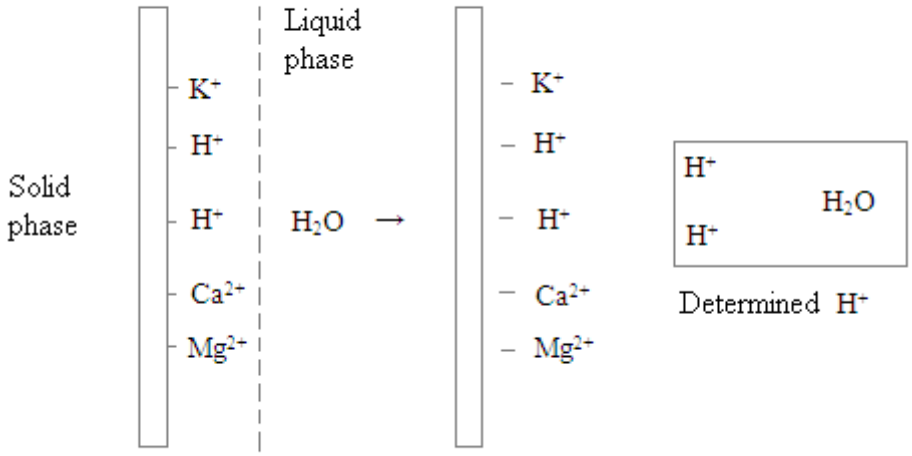
- active soil reaction (pH/H_2O)

- exchangeable soil reaction (pH/KCl)
- hydrolytic soil reaction (Ha; mmol H^+ . 100 g soil)

2.4.1 Determining the active soil reaction (pH/H_2O)

The active soil reaction indicates the concentration of hydrogen ions in water extract or soil solution. The primary principle for its determination is shown in the picture below (Fig. 10).

Fig. 10: The scheme for active soil reaction determination (pH/H_2O)



Laboratory equipment and reagents: analytical balance, beaker (50 ml), graduated cylinder, glass rod, laboratory spoon, pH-meter with combined electrode, distilled water

Work process:

Place 10 g of air-dried fine grained soil sample I into a beaker and add 25 ml of distilled water, and then boil and cool it down for 5 minutes to prevent CO_2 formation. Stir the suspension with a glass rod for 5 minutes and let it stand afterwards for at least 2 hours. The International standard allows a time of extraction from 2 to 24 hours. After the elapsed time, put the combined electrode-pH-meter into the suspension and record the result with an accuracy of one decimal place. The active soil reaction is then evaluated according to the table below (Tab. 7).

Tab. 7: Evaluating the active soil reaction

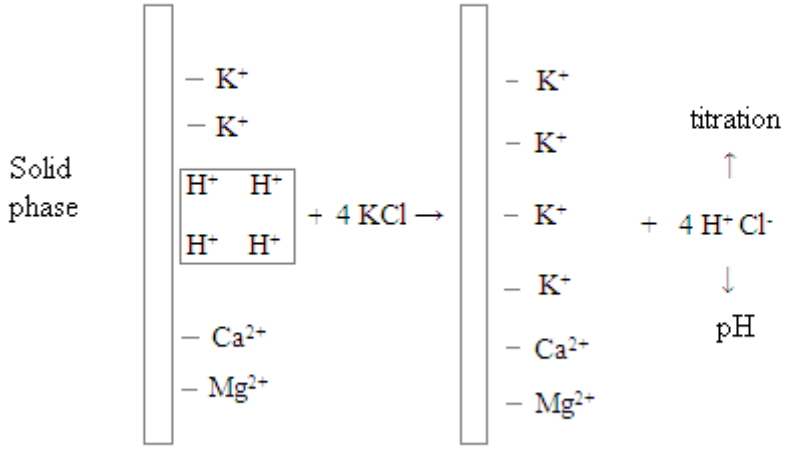
pH/H_2O	**Soil evaluation**
< 4,9	Strongly acid
5,0 – 5,9	Acid
6,0 – 6,9	Slightly acid
7,0	Neutral
7,1 – 8,0	Slightly alkaline
8,1 – 9,4	Alkaline
> 9,5	Strongly alkaline

2.4.2 Determining exchangeable soil reactions (pH/KCl)

An exchangeable soil reaction is defined as the ability of a soil to change the pH values of neutral salts (electrolytes). Hydrogen ions, which are bound by a sorption complex, are replaced by neutral salt ions that affect the soil. Solutions of KCl (1 mol/dm^3) or $CaCl_2$ (0.01 mol/dm^3) are usually used, and consequently the soil reaction is marked by the symbols pH/KCl or pH/$CaCl_2$ according to the appropriate reagent. The primary principle of the reaction is shown in the picture below (Fig. 11).

An exchangeable soil reaction is determined by pH measurement or leachate titration. The first technique will be used for our laboratory training. The exchangeable soil reaction reaches lower values when compared with the active one because hydrogen ions bound by sorption complex are determined together with free hydrogen ions in soil solution as well.

Fig. 11: A scheme representing the exchangeable soil reaction determination (pH/KCl)



Laboratory equipment and reagents: analytical balance, beaker (50 ml), graduated cylinder, glass rod, laboratory spoon, pH-meter with combined electrode, distilled water, 1 mol/dm^3 solution of KCl

Work process:

Place 10 g of air-dried, fine-grained soil sample I into a beaker and add 25 ml of 1 mol/dm^3 KCl solution. Stir the solution with a glass rod (app. 5 minutes) and allow it to stand until the next day (app. 24 hours). After the elapsed time, put the combined electrode-pH meter in the suspension and record the measured value (pH/KCl). The exchangeable soil reaction is evaluated afterwards according to the table below (Tab. 8).

Tab. 8: Evaluation the exchangeable soil reaction

pH/KCl	Soil evaluation
< 4,5	Strongly acid
4,6 – 5,5	Acid
5,6 – 6,5	Slightly acid
6,6 – 7,2	Neutral
> 7,3	Alkaline

2.4.3 Determining hydrolytic soil reactions (Ha; mmol H^+ . 100 g soil)

A hydrolytic soil reaction is defined as the ability of a soil to change the reaction of hydrolytic dissociable salts, whereas sodium or calcium acetates are usually used. Less dissociable acetic acid originates during the reaction, and the chemical equilibrium simultaneously shifts to the right side (12). For this reason, titration is used instead of pH measurement.

$$H - R \equiv Al + 2(CH_3COO)_2Ca + 3\ H_2O \leftrightarrow Ca = R = Ca + Al(OH)_3 + 4\ CH_3COOH \qquad (12)$$

Neutral salts displace only some of the hydrogen ions from a sorption complex during the exchangeable reaction because a high amount of H^+ inhibits the end of the reaction after equilibrium is established. For acetate, almost all hydrogen ions are displaced and only a small amount of H^+ nearly allows the reaction to finish (less dissociated acetic acid forms). For this reason, hydrolytic soil reactions provide higher values than exchangeable one.

In several cases, the opposite event can occur in which hydrolytic soil reactions yield lower values. This finding is caused by the soil sorption of acetic acid anions instead of hydroxide anions when soil solution is much more acid. Therefore, a determination of the hydrolytic soil reaction is stopped; it is used only for the sorption complex saturation determination according to Kappen (1929).

Laboratory equipment and reagents: PVC bottle with lid (500 ml), pipette (50 ml), Erlenmeyer beaker (250 ml), shaker, filtering funnel, filter paper, 1 mol/dm^3 sodium acetate solution, 0.1 mol/dm^3 NaOH, phenolphthalein, distilled water

Work process:

The reagents must be prepared first to produce 1 mol/dm^3 solution of sodium acetate. Weigh 136.08 g p.a. salt of CH_3COONa . 3 H_2O, which is dissolved in 1 litre of distilled water. The pH value must be 8.2 so that acetic acid, sodium hydroxide or phenolphthalein can be used to regulate it (light pink colour).

Weigh 40 g of soil into a PVC bottle, add 100 ml of sodium acetate, cover the bottle and let it shake for 1 hour. Filter the solution and remove 50 ml with a pipette into the Erlenmeyer beaker. Add 2 – 3 drops of phenolphthalein and titrate the solution against NaOH until a light pink colour is reached.

Hydrolytic acidity (H_a) is further calculated according to formula (13) and the results are evaluated according to the table (Tab. 9) below.

$$H_a = \frac{a*c(NaOH)*100*K}{g} \text{ [mmol(+)/100 g]} \quad (13)$$

a = NaOH consumption during titration (cm^3)
c (NaOH) = NaOH molar concentration (0.1 mol/l)
g = soil sample mass (g)
100 = corrected to 100 g of soil
K = coefficient of partial influence of acetate on a soil
K = 1.75 for sodium acetate
K = 1.5 for calcium acetate

Tab. 9: Evaluating the hydrolytic soil reaction

Ha (mmol(+)/100 g)	**Evaluation**
> 1.37	Very strong
1.37 – 0.92	Strong
0.92 – 0.63	Medium
0.63 – 0.29	Mild
0.29 – 0.17	Weak
< 0.17	Very weak

2.5 Determining carbonate contents

Carbonates represent a very important part of the soil mineral component. In soils, they are primarily present in a form of calcium carbonate ($CaCO_3$), and fewer are found in a form of magnesium carbonate ($MgCO_3$). The other forms are sporadically apparent in soils. Carbonates have a very important influence on soil properties, e.g., through sorption complex saturation or buffering capacity.

These compounds are of primary (parent rock) or secondary (fertilizers) origin in soil. In the first case, their content in a soil profile decreases towards the surface, which means that the highest content is recorded at lower parts of soil profile. In the second case, carbonates are present primarily in the surface layers.

All of the carbonate determination methods here are based on carbon dioxide (CO_2) release, which is caused by suitable acid treatment, in most cases by HCl (14). The result is explained in terms of the calcium carbonate content in a soil sample but it is important to remember that all of the carbonates and bicarbonates present in the soil sample are broken down, not only calcium carbonate. Several compounds decompose very slowly, only at higher temperatures; therefore, they cannot be determined by using the following methods.

Other gases (e.g., the hydrogen sulphide of anaerobic sulphide soils) can be released from soils when reacting with acids. In these cases, higher carbonate contents can be determined and that is why other methods of determination must be used. However, this is usually not a problem in agricultural land.

Soils with a pH value lower than 6.5 do not usually contain carbonates; the only exceptions are recent liming or the presence of barely soluble limestone.

$$CaCO_3 + MgCO_3 + 4\ HCl \rightarrow CaCl_2 + MgCl_2 + 2\ H_2O + 2\ CO_2 \quad (14)$$

2.5.1 Qualitative determinations of carbonates

The first very important step is to find if carbonates are present at all and at what content. Carbonates can be estimated according to the intensity of bubbling that is caused by released CO_2. In addition, the amount of soil sample to use for a quantitative determination is found this way.

Laboratory equipment and reagents: analytical balance, porcelain dish, laboratory spoon, HCl (10 %)

Work process:

Place 1 g of air-dried, fine-grained sample I into the porcelain dish with diluted HCl (10 %). The approximate amount of carbonates is determined according to the time and intensity of gas evolution, and evaluated according to the table below (Tab. 10).

Tab. 10: Qualitative determinations of carbonates in soil

Carbonate contents (%)	Gas evolution
< 0.3	None or scarcely any
0.3 – 1.0	Weak
1.0 – 5.0	Obvious
> 5.0	Intensive and long-lasting

2.5.2 Qualitative determinations of carbonates by using the Janek Calcimeter

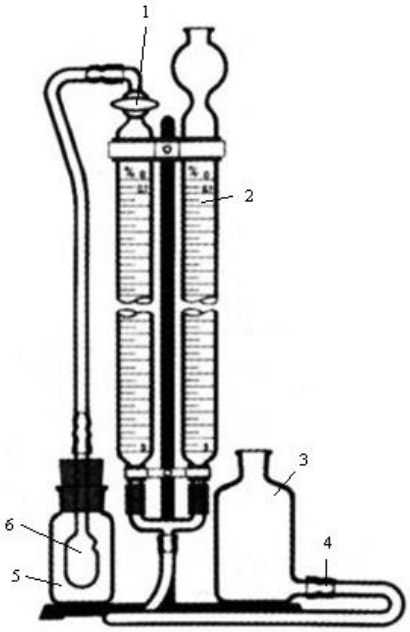
Carbonates are analysed by using diluted hydrogen chloride acid. The amount of CO_2 that is released can be determined in two ways, namely, volumetric and manometric as a percent of $CaCO_3$. The first method will be used for laboratory training.

Laboratory equipment and reagents: analytical balance, Janek Calcimeter, laboratory spoon, HCl (10 %), distilled water

Work process:

First, set a three-port valve of the Janek Calcimeter (Fig. 12) so that the lateral opening points downwards, which means that the eudiometric pipe is connected to the air. Raise the storage bottle containing distilled water and uncock the pusher so that both tubes are filled with water until the zero point is reached. Tighten the pusher to prevent the water levels in the tubes from falling. Weigh 2 to 20 g of fine grained soil sample I (in accordance with the result of the quantitative analysis) into the developer bottle. Add 15 ml of diluted HCl (10 %) into a special tank and turn the three-port valve about-face to connect the developer bottle with the air. Insert the special tank with HCl into the developer bottle and slowly mix the acid with soil until the gas evolution stops. Uncock the pusher again and allow the water levels in both tubes to equalize. Read the calcium carbonate content from the scale and evaluate it according to the following table (Tab. 11).

Fig. 12: Janek Calcimeter



Legend:

1 - free-port valve

2 - eudiometric pipe

3 - storage bottle

4 - pusher

5 - developer bottle

6 – tank

(Source: http:www.helago-cz.cz)

Tab. 11: Quantitative determinations of carbonates in soil

Carbonate contents (%)	Soil nomenclature
< 0.3	Soil without carbonates
0.3 – 3.0	Slightly limey
3.1 – 25.0	Limey
25.1 – 60.0	Marl
> 60.0	Calcareous

2.6 Soil organic matter

Soil organic matter underlies complicated processes, such as humification, mineralization and others, which means that it goes through continuous changes. The principle of humus content determination is organic carbon oxidation. Organic carbon in soils is investigated from qualitative and quantitative points of view, whereas the humus quality is often more important than its quantity. The methods are further divided into the dry-way and wet-way. The dry-way methods usually allow for complete oxidation; however, the wet-way methods generally give lower results. Nevertheless, these differences can be neglected for routine purposes. Organic carbon oxidation is performed either directly, from the soil sample, or indirectly, from soil solution by using various reagents according to the determined characteristics.

2.6.1 Quantitative humus content determinations

For quantitative methods, organic carbon can be determined by dry-way methods (annealing method and elemental analysis) and by dry-way methods (oxidometric method and spectrophotometric method).

2.6.1.1 Dry-way methods of determination

Annealing method

This method is direct. The oxidation is performed in a flame or an annealing furnace. The annealing temperature cannot be higher than 530 °C to prevent carbonate disintegration.

The disadvantage of this method is its constitutional clay water evolution, which is why it is used primarily for samples with high organic substance contents, in which

the determination mistake is supposed to be lower. Nevertheless, this method is only approximate.

Laboratory equipment and reagents: analytical balance, annealing pot, aluminous dryer, laboratory oven, laboratory spoon, annealing furnace, desiccator, crucible holder

Work process:

Place the sample of fine-grained soil I into the aluminous dryer and dry until a constant weight is reached. Simultaneously weigh the numbered annealing pot (value A) and fill it with 5 g of soil sample (value B). Put the pot with weighed soil into the annealing furnace for 1 hour. Remove it and put it in the dessicator for approximately 1 hour to reach the laboratory temperature and weigh again (value C). The percentage loss is counted afterwards from the weight difference (15).

$$OOU = \frac{B-C}{B-A} * 100 \quad [\%] \tag{15}$$

Elemental analysis

Elemental analysis is an indirect method that is instrumental to the determination of not only carbon but also other elements, e.g., hydrogen, sulphur, nitrogen and oxygen. This method's principle lies in soil sample combustion at high temperatures up to 1,800 °C. The combustion products are further separated and quantitatively determined by gas chromatography (GC) coupled to a detector (most often TCD – Thermal Conductivity Detector). The per cent composition of each individual element is set by a calibration factor calculation, which is determined during standard analysis in which sulphonyl amide typically serves as a standard.

2.6.1.2 Wet chemical methods of determination

Although there are several variations of wet chemical methods, they all involve organic carbon ($C_{org.}$) oxidation in the presence of sulphuric acid (H_2SO_4). The most common oxidant is usually potassium dichromate ($K_2Cr_2O_7$), which has replaced the previously used potash ($KMnO_4$). The amount of oxidised carbon can then be determined by the amount of CO_2 produced or by the extent of oxidant consumption.

Among some of the factors that can influence results from this method are oxidant concentration, H_2SO_4 concentration, temperature and duration of the oxidation. The most effective and widely used method is the modified Tjurin method, which provides up to 93 % of the combustion product.

Modified Tjurin method

The principle of the modified Tjurin method is the oxidation of organic carbon by an appropriate oxidant ($K_2Cr_2O_7$) in sulphuric acid medium. The amount of oxidant consumed can be determined by Mohr´s salt or by chromous sulphate reverse titration. This method proceeds according to the following reactions (16 – 18).

$$2\ K_2Cr_2O_7 + 8\ H_2SO_4 \rightarrow 2\ K_2SO_4 + 2\ Cr_2(SO_4)_3 + 8\ H_2O + 3\ O_2 \quad (16)$$

$$3\ O_2 + 3\ C \rightarrow 3\ CO_2 \quad (17)$$

$$K_2Cr_2O_7 + 7\ H_2SO_4 + 6\ Fe(NH_4)_2(SO_4)_2 \rightarrow K_2SO_4 + Cr_2(SO_4)_3 + 3\ Fe_2(SO_4)_3 + 6\ (NH_4)_2SO_4 + 7\ H_2O \quad (18)$$

Laboratory equipment and reagents: analytical balance, beaker (200 ml), watch-glass, laboratory oven, graduated cylinder (10 ml), magneto-electric mixing device, stirrer, Pt electrodes, galvanometer, burette, cooking capillary, chromous sulphate mixture (0.0667 mol/dm^3 $K_2Cr_2O_7$ in sulphuric acid), 0.1 mol/dm^3 $Fe(NH_4)_2(SO_4)_2$, diphenylamine, 85 % H_3PO_4, and distilled water.

Work process:

It is necessary to first prepare the reagents. For the chromous sulphate mixture preparation, 19.6148 g of $K_2Cr_2O_7$ is dissolved in 400 ml of distilled water. The solution is cooled slowly while 500 ml of concentrated H_2SO_4 is simultaneously added. Finally, distilled water is added to the solution until a volume of 1,000 ml is reached.

For the Mohr´s salt preparation, 39.21 g of $Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$ is dissolved in 300 ml of distilled water. The solution is then filtered into 20 ml of H_2SO_4 and diluted to a final volume of 1,000 ml with distilled water.

For the preparation of 100 ml of diphenylamine solution, 1 g of diphenylamine is dissolved in 85 % H_3PO_4 and then filled to volume with concentrated H_2SO_4.

The procedure continues by weighing 0.4 g of fine-grained soil sample II. For soils with low humus content, the amount of fine-grained soil increases, whereas that for organogenic soils decreases. Next, 10 ml of 0.0667 mol/dm^3 chromous sulphate mixture is added to the soil sample, which is then cover with a watch-glass and placed in the laboratory oven for 45 minutes (125 °C). After the elapsed time, the sides of the beaker as well as the watch-glass are washed with distilled water. It is necessary to regulate, using distilled water, the volume of solution used in the potentiometric titration to enable the stirrer to operate under the Pt electrodes. When the electrodes are ready for use, the magneto-electric mixing device is turned on, allowing the electric current to flow, and the solution can be titrated by 0.1 mol/dm^3 $Fe(NH_4)_2(SO_4)_2$. The progress of the titration is followed by a galvanometer. Before the end of the titration, the pointer of the galvanometer shows a temporary displacement. A permanent displacement indicates the establishment of an equilibrium state. Finally, using the Mohr´s salt consumption, the results can be calculated.

Next, 0.0667 mol/dm^3 $K_2Cr_2O_7$ is titrated on 8 drops of indicator (diphenylamine) in case of reverse titration. A colour transition from green-grey to blue/blue-grey is observed. To emphasise the colour transition, 3 ml of 85 % H_3PO_4 is added before the titration. The consumption of the chromous sulphate mixture is equivalent to the amount of $K_2Cr_2O_7$ used in the carbon oxidation.

The titre is not steady as a result of Fe^{2+} ions; therefore, an assessment of a salt factor is necessary. The procedure is similar to that used for the soil samples except that several cooking capillaries are to prevent latent boiling. The factor is further calculated as the average value of three parallel measurements.

Finally, the appropriate calculations (19 – 22) and interpretations (Tab. 12) are performed.

a) Mohr´s salt factor calculation:

$$f = b / a \tag{19}$$

b = theoretical consumption of 0.1 mol/dm^3 $Fe(NH_4)_2(SO_4)_2$ for the purpose of titration of 10 ml of 0.0667 mol/dm^3 $K_2Cr_2O_7$

a = real consumption for the purpose of blind sample

According to formula (19), potassium dichromate reacts with Mohr´s salt in the following manner:

1 mol $K_2Cr_2O_7$ .....6 mol $Fe(NH_4)_2(SO_4)_2$

1 ml 0.0667 mol/dm^3 $K_2Cr_2O_7$ .....4 ml 0.1 mol/dm^3 $Fe(NH_4)_2(SO_4)_2$

0.0667 mmol $K_2Cr_2O_7$ .....6 * 0.0667 mmol $Fe(NH_4)_2(SO_4)_2$

Four millilitres of Mohr´s salt is used for titration of 1 ml of potassium dichromate. Consequently, the theoretical consumption (b) will be 40 in the case of 10 ml.

b) C_{org} calculation:

- titration by Mohr´s salt

$$C_{org} = (12 - 0{,}3 * S' * f) \cdot 100/N \quad [\%] \tag{20}$$

S´= consumption of Mohr´s salt during titration [ml]

f = factor of Mohr´s salt

N = sample weight [mg]

$$\% \text{ humus} = C_{org} * 1.724 \tag{21}$$

1.724 = Welte´s converting coefficient (results from the 58 % carbon content in humus)

- reverse titration by chromous sulphate mixture

$$C_{org} = 120 * R/N \quad [\%] \tag{22}$$

R = consumption of 0.0667M $K_2Cr_2O_7$ during titration

N = sample weight [mg]

Tab. 12: Evaluation of humus content

C_{org} content (%)	**Humus content (%)**	**Description**
< 0.6	< 1	Very low
0.6 – 1.1	1.0 – 2.0	Low
1.1 – 1.7	2.0 – 3.0	Medium
1.7 – 2.9	3.0 – 5.0	High
> 2.9	> 5.0	Very high

2.6.2 Qualitative humus content determination

Several methods for the qualitative determination of humus exist; however, none of them describe humus characteristics from all points of view. Among the most important features considered include the degree of polymerisation and humification, aliphatic and aromatic character, sorption, mineral structures, colloid properties and chemical composition. However, the ratio between humic and fulvic acids (HA:FA) and carbon and nitrogen (C:N) as well as the optical properties of alkali extracts of humic compounds are also common features of humus quality determination and colour quotient ($Q_{4/6}$) calculation.

2.6.2.1 Spectrophotometric determination

The main principle lies in the determination of the polymerisation/dispersity degree of alkali solutions of humic compounds in terms of absorbance estimation by spectrophotometric measurements. This method results from the Lambert-Beer Law, which describes the relationship between the absorption intensity of a compound dispersed in a non-absorbing environment and the initial intensity of monochromatic light, layer thickness and concentration.

Transmission (23), absorption (24) and absorbance (25) can be determined during spectrophotometric measurements.

$$T = \frac{I}{I_0} = 10^{-\varepsilon c l} \tag{23}$$

T = transmission (ranges between 0 and 1)
I = intensity of radiation passing the cuvette
I_0 = intensity of incident radiation
ε = molar absorption capacity
c = molar concentration
l = layer thickness

$$B = 1 - T \tag{24}$$

B = absorption (ranges between 0 and 1)

$$A = \log \frac{1}{T} = \log \frac{I_0}{I} = \varepsilon c l \tag{25}$$

A = absorbance (ranges between 0 and ∞)

Laboratory equipment and reagents: spectrophotometer, analytical balance, Erlenmeyer beaker, shaker, centrifuge, solutions of humic substances, extracts, and distilled water.

Work process:

A solution of humic substances is prepared by extraction of 0.05 mol/dm^3 sodium pyrophosphate ($Na_4P_2O_7$) with the ratio of soil:extract = 1:20 is held constant. A soil sample (5 g) is weighed and placed in an Erlenmeyer beaker, a solution of sodium pyrophosphate is added, and the solution is allowed to shake for 1 hour. Then, the suspension is separated and the pure solution is modified by extracts to ensure that the absorbance will be 0.900 at a wavelength of A = 400 nm. This solution is measured on a spectrophotometer at a minimum of 5 different wavelengths of visible light (λ = 400, 450, 500, 550 a 600 nm). As a comparison solution, H_2O, $Na_4P_2O_7$, and NaOH can be used.

To eliminate inaccuracy, a least squares method is used. These results are instrumental to the construction of absorbance curves (Fig. 13) and absorbance semi-logarithmic lines (Fig. 14) as well as colour quotient $Q_{4/6}$ calculations (26).

$$Q_{4/6} = \frac{A_{400}}{A_{600}} \qquad (26)$$

$Q_{4/6}$ = colour quotient (λ = 400 to 600 nm)
A_{400} = absorbance (λ = 400 nm)
A_{600} = absorbance (λ = 600 nm)

Fig. 13: Absorbance curves

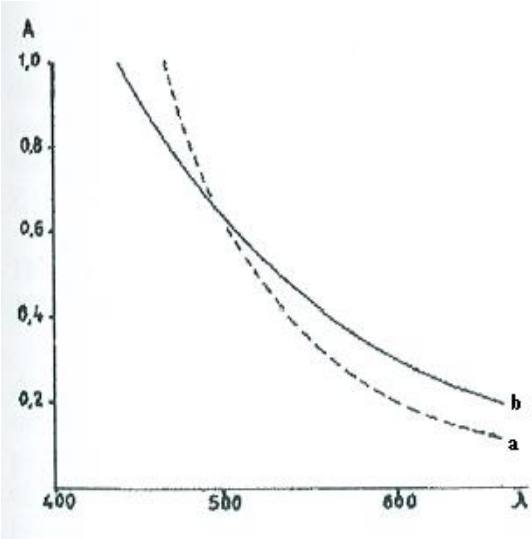
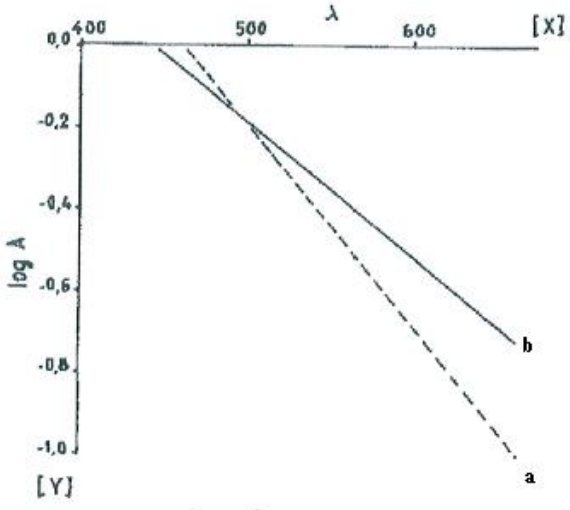


Fig. 14: Absorbance semi-logarithmic lines



From the results of the absorbance curves, steeper runs were found to indicate lower degrees of condensation and polymerisation (see curve “a” in Fig. 13). Conversely, in the case of the semi-logarithmic lines, lower runs indicate higher degrees of condensation and polymerisation (see curve “b” in Fig. 13). The colour quotient is indirectly proportional to the degree of condensation and polymerisation, i.e., the lower the quotient, the more polymerised and stable the humic substances are.

2.6.3 Cellulase activity determination according to Christensen and modified by Grunda

Soil organisms dispose of enzymatic apparatus that fission cellulose, which is the main component of a plant’s body. Cellulose present in dead organic matter is decomposed by two groups of organisms: saprophytic organisms, which are able to produce cellulolytic enzymes, and symbiotic organisms, which live in the digestive tract of mites, ants and others.

Although determination according to Christensen and modified by Grunda cannot evaluate the quality of enzymes, it is a suitable method for total microbial activity determination.

Laboratory equipment and reagents: analytical balance, filter paper, annealing pot, laboratory tweezers, casement cloth, laboratory oven, desiccator, annealing furnace, pencil, and plastic bag.

Work process:

A disc measuring 10x10 cm is made from filter paper and then numbered using a pencil, placed in a laboratory oven (2 hours, 120 °C), and allowed to cool in a desiccator. The filter paper is then removed from the desiccator using laboratory tweezers and weighed (value A). Afterward, the weighed filter paper is placed on a casement cloth (12x24 cm) and completely covered. The discs individually wrapped in casement cloth are then placed in a plastic bag and taken into a field where they are placed in the soil approximately 5 – 10 cm below the surface.

After 7 days, the discs are removed from the soil and placed back in the plastic bag. In the laboratory, all pieces of filter paper are removed using laboratory tweezers and placed

in marked annealing pots, being careful not to allow roots and other material to enter the pots. The pots containing the filter paper are dried in laboratory oven for 2 hours at 120 °C. After the elapsed time, the pots are removed , placed in a desiccator, weighed (value B) and placed in an annealing furnace for 1 hour (500 °C). The samples are then allowed to cool in a desiccator before being weighed once more (value C). Finally, the appropriate calculations (27) and results interpretation (Tab. 13) are conducted.

$$\text{Cellulase activity} = \frac{A-B-C}{At} \quad [-] \tag{27}$$

t = time of exposition in soil [weeks]

Tab. 13: Cellulase activity evaluation

Cellulase activity	Activity evaluation
0	None
0 – 1	Very weak
1 – 2	Weak
2 – 5	Medium
5 – 10	Strong
> 10	Very strong

2.6.4 Soil enzyme activity determination

The determination of soil enzyme activity is one of the most important factors monitored for the quality of soil microorganisms. The evaluation provides data on the soil environment and metabolic preference of selected microbial species. This method can also be used for soil biochemical processes and soil quality studies. Soil enzymes catalyse the metabolic activity of microbes; however, most of them are selective in that they catalyse only one particular substrate. For this reason, enzyme activity is being evaluated by synthetic enzymes, from which the detectable component is released. Because enzyme activity strongly dependent on temperature, the use of incubation apparatus is necessary.

2.6.4.1 Determination of soil dehydrogenase activity

The main principle is the reduction of triphenyltetrazolium chloride (TTC) to triphenylemazane (TTF). The soil sample reacts with a TTC solution, whereas TTF is extracted by acetone and evaluated by means of spectrophotometry (wavelength of 546 nm).

Laboratory equipment and reagents: analytical balance, conical centrifuge tubes, vortex, laboratory spoon, automatic pipette, shaker, centrifuge, glass cuvette, spectrophotometer, Tris-HCl buffer solution, TTC solution, acetone, and distilled water.

Work process:

The appropriate solutions must be prepared in advance. TRIS buffer solution is prepared from 12.1 g in 1 litre of distilled water and the pH value is adjusted to 7.6. TTC solution consists of 1 g of triphenyltetrazolium in 1 litre of TRIS buffer.

Approximately 2 g of the soil samples is weighed into conical centrifuge tubes and the accurate backfill is recorded. Three tubes must be used: two as replicates and one as a control. TTC solution (5 ml) is added to the repetition tubes and 5 ml of TRIS is added to the control tube. The tubes are then closed and mixed using a vortex during which time they are incubated at a constant temperature of 25 °C for one week. It is also necessary to prevent the tubes from being exposed to light.

After the elapsed time, 16 ml of acetone is added and the tubes are allowed to mix via vortex for one hour. The tubes are then centrifuged (4,000 rpm, 8-10 minutes) and transferred to a glass cuvette for absorbance measurement ($\lambda = 546$ nm) against pure acetone (blank).

2.6.4.2 Determination of soil enzymes via direct incubation

The principle of this determination is the dissolution of synthetic enzymes in water or an appropriate buffer. The presence of enzymes is further evaluated by spectrophotometry at a wavelength of 400 nm.

Laboratory equipment and reagents: analytical balance, laboratory spoon, micro-tubes (Eppendorf), centrifuge, vortex, spectrophotometer, micro-titration plate, automatic pipette, substrate, 1 M sodium carbonate, and distilled water.

Work process:

It is necessary to prepare particular substrates in advance. These substances vary according to enzyme type are listed along with their concentrations and backfills in the table below (Tab. 14).

Tab. 14: Determination of soil enzymes – backfill and substrate

Enzyme	Substrate	Concentration	Backfill (on 10 ml)
Phosphatase	4-nitrophenyl phosphate disodium salt hexahydrate	0.001667 g/l	0.01667 g
Deaminase	L-alanine 4-nitroanilide hydrochloride	1.10^{-3} mol/l	0.00246 g
Arylsulphatase	Potassium 4-nitrophenyl sulphate	$3{,}333.10^{-3}$ mol/l	0.00858 g
Glucosidase	4-nitrophenyl β-D-glukopyranoside	1.10^{-3} mol/l	0.00301 g

The soil sample is weighed (0.05 g) and placed in a micro-tube, ensuring that an accurate backfill is recorded. Then, 0.5 ml of a particular substrate is added. It is necessary to set the timer before the substrate is added because the time of the soil and substrate reaction varies for individual enzymes: 30 minutes for phosphatase, 3 – 4 hours for deaminase, 4 hours for arylsulphatase and 5 hours for glucosidase. Then, the samples are mixed and allowed to stand in the room at a stable temperature for the given time period.

After the elapsed time, 0.25 ml of enzyme inhibitor (1 M sodium carbonate) is added and the samples are centrifuged for 5 minutes (13,000 rpm). Then, 300 µl of the sample is placed in a micro-titration plate to measure the absorbance (wavelength of 400 nm).

The control samples are determined by following the same procedure, except that water is added instead of 1 M sodium carbonate and the reaction time is 3 hours.

It is also necessary to measure a control to ensure that the substrate does not spontaneously disintegrate. For this purpose, 200 µl of the substrate is mixed with 100 µl of sodium carbonate. If the absorbance of this mixture is higher, it must be subtracted from the results.

Finally, the differences between absorbance values (28), as well as enzyme activity (29), are calculated.

$$\Delta A = A_v - A_k * \frac{m_v}{m_k} \qquad (28)$$

A_v, A_k = absorbance of the sample and the control, respectively

m_v, m_k = mass of the sample and the control [g], respectively

$$U = \frac{1000 * \Delta A * V}{\varepsilon * t * m_v} \quad [\mu mol/min/g] \qquad (29)$$

V = volume of the liquid in Eppendorf tube (0.75 ml)

ε = extinction coefficient (11,600 $M^{-1}cm^{-1}$)

t = reaction time [min]

2.7 Soil sorption, sorption complex

Soil sorption represents one of the most important chemical processes that expresses a soil's ability to bind substances in a dispersed medium. Soil colloids, which create part of the solid phase, or so-called soil colloidal complex, also participate in sorption. Soil sorption can be divided into two basic groups: adsorption (binding on the outer or inner surface) and absorption (entering the internal portion).

The sorption complex has a considerable influence on soil dynamics and physical conditions and it also participates in plant nutrition. The conditions and properties of sorption complex directly affect sorption capacity, soil reaction and porosity, whereas they indirectly affect soil structure, water and air regime, biological activity and cultivation.

2.7.1 Cation exchange capacity

Cation exchange capacity (CEC) represents the amount of cations that can be bound by soil depending on an appropriate pH value. The exchange of cations is caused by the negative charge of colloid clay and humic particles. The negative charge consists of two parts: permanent charge and variable charge, which depends on pH.

Permanent charge forms as a result of isomorphic substitution of Al^{3+} by Si^{4+} in the tetrahedral layer, and of Mg^{2+} (and eventually Fe^{2+}) by Al^{3+} in the octahedral layer of clay minerals. The variable charge originates either during dissociation of the hydroxyl groups of phenols and carboxyl groups, or on the aluminosilicate layer, as well as on edges of laminated silicates and silane groups.

As mentioned above, variable charge is pH dependant. When the pH value is low, aluminium hydroxyl ions, which block negative charge, are sorbed. These ions are precipitated in the form of $Al(OH)_3$ when the pH value increases, thus the negative charge is released.

Several complications can arise during the determination of the cation exchange capacity. This determination assumes that the charge of the cations is known, whereas many polyvalent cations form hydroxyl ions (e.g., montmorillonite adsorbs large amounts of $CaOH^+$ and $MgOH^+$). From this point of view, the cation exchange capacity will be 5 % overestimated (pH = 7) if only the cations Ca^{2+} and Mg^{2+} are studied.

Another complication is related to the parallel presence of both positive and negative charges, which are supposed to be measured separately in concentrated solutions of strong electrolytes. When the concentration is low, electrical double-layers expand as much as they overlay and the charges are neutralised. Then, the cation exchange capacity value is equal to the resultant charge, which can be considerably lower than the total negative charge. Because the positive charge is neutralised at pH 7, lower concentrations should be used during CEC determination.

In the case of acid soils, two reactions of bases and acid soil should be taken into account. The first one (30) is responsible for neutralisation of the negative charge by aluminium cations on the surface of clay minerals. The reaction of OH^- causes the formation of $Al(OH)_3$, which indicates that surface aluminium cations are replaced by basis and the total charge does not change. The second reaction (31) describes ionisation of organic acids and phenols, which cause an increase in negative charge.

$$Al^{3+} + 3\ OH^- \rightarrow Al(OH)_3 \tag{30}$$

$$ROH + H_2O \rightarrow RO^- + H_3O^+ \tag{31}$$

The cation exchange capacity is usually marked with a T symbol and several methods are being used for its determination. The determination according to Mehlich will be described later in more detailed, whereas the other methods are mentioned and their backgrounds summarised in the following section.

2.7.1.1 Methods of cation exchange capacity determination

As mentioned above, several methods are used for cation exchange capacity determination. In this chapter, these methods will be specified along with their main principles.

1) Washing with concentrated acid (e.g., HCl) and titration via $Ba(OH)_2$ (pH < 7) or NaOH (pH < 8.5). The analysed sample is then saturated with H^+ and Al^{3+} and the amount of hydroxide (mmol) required for neutralization is determined.
2) Summing methods. Exchangeable cations are displaced by a salt solution. The cation exchange capacity represents the sum of these cations. Both buffer and non-buffer salts can be used in this method. In the case of buffer salts, only Ca, Mg, Na and K can be determined. Exchangeable acidity, determined separately at a buffering solution pH level, must be added to their sum. Using non-buffer salt enables determination of Al^{3+}, Ca^{2+}, Mg^{2+}, Na^+, K^+ and sometimes Mn^{2+} in the extracts. Their sum is equal to the CEC at a natural soil pH level.
3) Direct displacement of saturation salts. The index cation can be displaced directly by another salt (e.g., KNO_3) when the salt solution (e.g., NH_4Cl) is used on the exchangeable cations displacement and on the negative charge saturation by the index cation (NH_4^+). NH_4^+ and Cl^- are further determined in the resulting extract and the cation exchange capacity is equal to their difference.
4) Index cation displacement. Soil can be rinsed of excessive amounts of saturation salts when the sorption complex is saturated by the index cation. The index cation is displaced and evaluated during this process. Both neutral as well as alkaline salts can be used. For neutral salts (pH = 7), the soil is saturated by 1 mol/dm^3 CH_3COONH_4 and its excess is washed by alcohol (to prevent hydrolysis). Adsorbed NH_4^+ can be determined either by airflow displacement (5 % Na_2CO_3 is added), or by washing (when NaCl solution is used, NH_4^+ is determined via the process of distillation and consequential titration). This method is favourable, especially when the simultaneous determination of exchangeable cations is necessary. The second method is to use 1 mol/dm^3 CH_3COONa, a quick and routine method that provides satisfactory results mainly when saline soils are concerned, or a buffer of $BaCl_2$ and $MgSO_4$.
5) Ion exchanger utilisation. The principle of this method lies in the shaking of the soil, water and insoluble ion-exchangeable resin, saturated with NH_4. The resin is later separated and the remaining soil solution is distilled with NaCl and MgO. Exchangeable cations can be dissolved and evaluated when using the appropriate method of ion exchanger washing.
6) Conductometric titration. Hydrogen ions of less dissociated parts are neutralised using lye during the process of titration. Before the saturation point is reached,

the conductivity begins to rise and the amount of titre solution at the flex point marks the soil sorption capacity.

7) Radioisotope utilisation. The concentration of the electrolyte is reduced when the soil is saturated by an index cation. Then, the suspension is marked by radioisotopes and the index cation concentration is evaluated. The radiometric measurement is used for isotope distribution between the two phases.

2.7.1.2 Determination of cation exchange capacity according to Mehlich III

The main principle of this method lies in soil extraction by an acidic solution that contains ammonium fluoride. Ammonium nitrate, which has a positive influence on potassium, magnesium and calcium desorption, is also present in the solution. Acid reaction of an eductive solution proceeds by means of acetic and nitric acids. The presence of ethylenediaminetetraacetic acid (EDTA) provides release of nutritive essential elements.

Laboratory equipment and reagents: analytical balance, rotary shaker, flame photometer (eventually AAS), filter device (laboratory stand with holders and separating funnel), beaker (250 ml), filter paper, PE bottles with lid (250 ml), laboratory spoon, nitric acid (65 %), acetic acid, ammonium fluoride, ethylenediaminetetraacetic acid (EDTA), and demineralised water.

Work process:

The extraction solution must first be prepared in which the following chemicals are necessary:

- Nitric acid (65 %).
- Concentrated acetic acid.
- Stock solution of ammonium fluoride and EDTA: dissolve 139 g of ammonium fluoride and 73.5 g of ethylendiaminotetraacetic acid in 750 ml of demineralised water. After complete dissolution, the volume is brought up to 1,000 ml.

The extraction solution is prepared by dissolution of 80 g of ammonium nitrate in 3,000 ml of demineralised water. Then, 16 ml of ammonium fluoride and EDTA solution, 46 ml of concentrated acetic acid and 3.3 ml of nitric acid are added to the solution. Demineralised water is then added until a final volume of 4,000 ml is reached.

The procedure continues by the process of extraction. The fine-grained soil sample I (10 g) is weighed into PE bottles, to which 100 ml of the prepared extraction solution is added. The bottles are shaken for 5 minutes (50 turns/minute) on a rotary shaker. After the process of shaking, the suspension is filtered through dense filter paper.

AES-ICP methods are used for measurement, the results of which are evaluated in the following table (Tab. 15).

Tab. 15: Cation exchange capacity and sorption complex evaluation

Cation exchange capacity (CEC)	**H (mmol(+)/100 g)**	**Sorption complex**	**V (%)**
Very high	> 30	Fully saturated	100 – 90
High	30 – 25	Saturated	90 – 75
Medium high	24 – 18	Less saturated	75 – 50
Medium low	17 – 13	Unsaturated	50 – 30
Low	12 – 8	Extremely unsaturated	< 30
Very low	< 8		

2.7.2 Anion exchange capacity

Anion exchange capacity (AEC) is connected to the presence of alkaline colloids with positive charges. Generally, the AEC is lower than the CEC and is strongly dependant on soil pH and salts concentration. The AEC increases with soil acidity in correlation with the rise of the positive charge of soil colloids, mainly hydrated sesquioxides. If different anions are present, selectivity will assert its influence (SiO_4^{3-} > PO_4^{3-} >> SO_4^{2-} > Cl^-). The anion exchange capacity is determined for phosphates in most cases.

2.7.2.1 Determination of anion exchange capacity according to Mehlich

The main principle of this method lies in the washing of cations from soil via triethanolamine and consequent saturation by calcium chloride. After the addition of phosphoric acid, the amount of extractable and adsorbed phosphorus is further determined, where their sum expresses the adsorption capacity of anions.

Laboratory equipment and reagents: analytical balance, photometer, pH meter, laboratory spoon, centrifuge, cuvettes (100 ml), laboratory oven, shaker, beakers (150 ml and 200 ml),

glass rod, volumetric flask (50 ml), laboratory stand, funnel, pipette, washing pipe, filter paper, 2 mol/dm^3 TEA, $BaCl_2$ (5 %), 0.3 mol/dm^3 $CaCl_2$, 0.01 mol/dm^3 H_3PO_4, 0.03 mol/dm^3 NH_4F in 0.1 mol/dm^3 HCl, solution of NH_4VO_3, $HClO_4$ (60 %), solution of $(NH_4)_2MoO_4$, standard solution of phosphate, ethanol (95 %), and distilled water.

Work process:

The following reagents must first be prepared:

- 2 mol/dm^3 solution of TEA: 90 ml of TEA is brought to a final volume of 1,000 ml using distilled water while the pH is regulated via HCl (pH = 8.1). Then, the solution is diluted to 2,000 ml by the addition of 2,000 ml of distilled water mixed with 100 g of $BaCl_2$. $2H_2O$. The resulting pH value should be 8.0 (if not, adjust using a saturated solution of $Ca(OH)_2$).
- 0.01 mol/dm^3 solution of H_3PO_4: prepare 0.93 g/dm^3.
- 0.03 mol/dm^3 NH_4F in 0.1 M HCl: 1.11 g of NH_4F is added to 1 litre of 0.1 mol/dm^3 HCl.
- Solution of NH_4VO_3: 2.345 g of NH_4VO_3 anhydride is dissolved in 400 ml of hot distilled water. Then, 17 ml of $HClO_3$ (60 %) is added before being diluted to 1,000 ml.
- Solution of $(NH_4)_2MoO_4$: 25 g of $(NH_4)_2MoO_4$ is dissolved in 400 ml of distilled water (approximately 50 °C). The solution is then filtered, diluted to 500 ml, and stored in brown glass bottles.
- Standard solution of phosphate: 0.2195 g of dried KH_2PO_4 (40 °C) is dissolved in 500 ml of distilled water and 25 ml of 3.5 mol/dm^3 H_2SO_4. The solution contains 50 μg/cm^3 of P after dilution to 1 litre.

Once all of the reagents are prepared, 10 g of fine-grained soil sample I is weighed and washed in a washing pipe (first with 100 ml of TEA, later six times with ethanol). Then, 100 ml of 0.3 mol/dm^3 $CaCl_2$ is used for washing, followed again by ethanol washing (6x). The soil is then dried in a laboratory oven (12 hours, 45 °C). Approximately 0.2 mmol of the sorption capacity value is weighed into cuvettes, 20 ml of 0.01 mol/dm^3 H_3PO_4 is added, and the samples are placed in a shaker for 30 minutes, followed by centrifugation (5 minutes, 250 turns/minute). The phosphorus is then determined in 1 ml aliquots. The amount of sorbed P is calculated from the difference between the original and determined amounts of phosphorus.

For the separate sample of original soil, the phosphorus is extracted by means of NH_4F in an HCl solution (soil:solution ratio = 1:20). The amount of P in the extract is then determined by the following procedure. An aliquot (containing 0.15 – 1.0 mg of P) is removed and placed in a volumetric flask, 5 ml of 60 % $HClO_4$ is added and the entire volume is diluted to 30 ml using distilled water. Then, 3 – 4 ml of $HClO_4$ is added so that the pH value equals 8.0. At the appropriate pH value, 5 ml of NH_4VO_3 solution is then added to the solution. It is necessary to filter off any precipitated SiO_2. Afterward, 5 ml of $(NH_4)_2MoO_4$ solution is added and the entire volume is brought to the scale line using distilled water. The absorbance (λ = 470 nm) is measured after 30 minutes. The range of the calibration graph is 0 – 20 $\mu g/cm^3$ P. Finally, the anion exchange capacity is calculated according to the formula (32) below.

$$AEC = \text{extracted P} + \text{adsorbed P} \quad [\text{mmol}/100\ \text{g of soil}] \tag{32}$$

2.8 Iron in soil

In soils, iron is present in igneous rocks, biotic micas and Fe-Mg silicates. In terms of its determination in soil, total iron and free iron oxides (Fe^{2+} and Fe^{3+}) can be distinguished. Total iron can be determined via X-ray fluorescence analysis or spectral methods. It is first necessary to disintegrate the sample by means of Na_2CO_3 fusion or HF. Although fusion is advantageous because other elements can also be determined in this manner, its low accuracy is a major disadvantage. Disintegration by HF provides more accurate results and is much more suitable for determining the iron content only. The principle of free iron oxide determination relies on reduction by means of various extract reagents.

2.8.1 Spectrophotometric determination of total iron content

The main principle of colorimetric determination is sample disintegration by means of HF and subsequent reduction via hydroxylamine-hydrochloride. The resulting red colouration leads to the absorbance determination.

Laboratory equipment and reagents: analytical balance, platinum pot with lid (30 ml), sand bath, burner, laboratory cook, glass rod, laboratory spoon, spectrophotometer, volumetric flasks (50 ml, 100 ml and 250 ml), pipettes, HF (48 %), HNO_3 (70 %), $HClO_4$ (70 – 72 %), 6 mol/dm^3 H_2SO_4, 1 mol/dm^3 and 5 mol/dm^3 ammonium acetate, 6 mol/dm^3 HCl, hydroxylamine-hydrochloride (10 %), orthophenanthrolin reagent, orthophenanthrolin monohydrate, 100 ppm Fe standard solution, $Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$, and distilled water.

Work process:

The following reagents must first be prepared:

- 6 mol/dm^3 H_2SO_4: 570 ml of concentrated H_2SO_4 is added to 1 litre of distilled water.
- 6 mol/dm^3 HCl: 534 ml of concentrated HCl is added to 1 litre of distilled water.
- Hydroxylamine-hydrochloride (10 %): 10 g of hydroxylamine-hydrochloride is added to 90 ml of distilled water.
- Orthophenanthrolin reagent: 0.3 g of orthophenanthrolin monohydrate is dissolved in distilled water (80 °C) and diluted to a volume of 100 ml.
- Standard solution of Fe (100 ppm Fe): 0.7022 g of raw $Fe(NH_4)_2(SO_4)_2 \cdot 6\ H_2O$ is dissolved in distilled water, warmed and dilute to 1,000 ml.

Fine-grained soil sample II (0.5 g) is weighed into a platinum pot. Several drops of distilled water, 5 ml of HF and 0.5 ml of $HClO_4$ are added and the pot is heated on a laboratory cook. When $HClO_4$ vapours appear, the pot is cooled and another 5 ml of HF are added. Then, the pot is transferred to a sand bath and is almost completely covered by a lid. To let its contents evaporate, the pot is heated to 200 – 250 °C. After cooling, 2 ml of distilled water and several drops of $HClO_4$ are added. The entire procedure is repeated. If there are remnants of organic matter on the sides and lid of the pot, they are allowed to burn through via a burner. After cooling, 5 ml of 6 mol/dm^3 HCl and 5 ml of distilled water are added and the pot is warmed above the burner. If the sample does not dissolve completely, another 5 ml of HF and 0.5 ml of $HClO_4$ can be added. Finally, 5 ml of 6 mol/dm^3 H_2SO_4 is added and the pot is heated to 250 °C for 15 minutes. After the elapsed time, the pot is cleaned by means of a glass rod, which is washed with 5 ml of 6 mol/dm^3 H_2SO_4. Simultaneously, the blank sample is also analysed.

For preparation of a calibration curve, 0.5, 15, 25, 35 and 45 ml of the solution with 5 ppm Fe are placed into volumetric flasks (100 ml). After shaking, 2 ml of hydroxylamine-hydrochloride and 2 ml of orthophenanthrolin reagent are added. Then, 1 mol/dm^3 ammonium acetate is added dropwise until a bright orange or red colour appears. An additional 3 ml of ammonium acetate is added before filling to the scale line with distilled water. The wavelength for the spectrophotometry analysis is 510 nm. A calibration graph is generated from measured values. The entire volume of the platinum pot is transferred to a volumetric flask (250 ml) using 20 ml of 6 mol/dm^3 HCl. The flask is then filled to the scale line with distilled water and stirred.

In a 50 ml volumetric flask, 1 ml of the resulting mixture is added by means of a pipette, followed by 2 ml of 5 M ammonium acetate and 1 ml of hydroxylamine-hydrochloride. After shaking, 1 ml of orthophenanthrolin reagent and 0.5 ml of 6 mol/dm^3 HCl are added. The solution is again diluted with distilled water and the pH value is measured. If the pH does not range between 3 and 5, 1 ml of burned sample from the beaker, in which the above-mentioned chemicals were added except for 6 mol/dm^3 HCl, can be added.

The solution is titrated with 6 mol/dm^3 HCl and the pH value must be measured simultaneously (final value must equal 4). Finally, the solution is analysed via spectrophotometry and the iron content in ppm is evaluated. The amount of iron is further calculated according to the formula (33) below.

$$\text{Amount of Fe} = \frac{(a-b)*1.25}{m} \quad [m] \tag{33}$$

a = amount of Fe in solution [ppm]

b = amount of Fe in blank sample [ppm]

m = sample weight [g]

2.8.2 Determination of free iron oxides

The principle of determination involves the use of citric buffer (pH 4.75), which enables fast extraction without sulphur precipitation. After the reaction with $Na_2S_2O_4$, the filtrate is burned by perchloric acid and the Fe and Al contents are analysed.

Laboratory equipment and reagents: analytical balance, sieve (mesh diameter = 0.15 mm), laboratory spoon, cuvettes (15 ml), beakers (100 ml), water bath, rubber cork, citric buffer, $Na_2S_2O_4$ powder, concentrated $HClO_4$, 0.1 mol/dm^3 HCl, citric acid, sodium citrate, and distilled water.

Work process:

First, the reagents must be prepared. Specifically, the citric buffer consists of 10.5 g/l of citric acid monohydrate and 147 g of sodium citrate. Then, 0.5 g of the soil sample, sieved through a sieve with a mesh diameter of 0.15 mm, is weighed. The soil is transferred to cuvettes with 10 ml of citric buffer and 0.5 g of $Na_2S_2O_4$. The cuvettes are corked and shaken in a water bath for 30 minutes at 50 °C. Each aliquot is burned in the beaker by $HClO_4$ when centrifuged and allowed to almost completely dry. The residues are placed into 0.1 mol/dm^3 HCl. the volume is standardised (50 ml) and the Fe content is evaluated by means of AAS or colorimetric methods.

2.9 Aluminium in soil

Aluminium is the most widespread metal on Earth and ranks among the most occurring elements in soil, besides oxygen and silica. Two forms of aluminium have been found in soil: organic and inorganic. Organic forms of aluminium are related to the presence of organic acids, especially those with high molecular weight (humic and fulvic acids). Inorganic forms of aluminium are present in 250 minerals (e.g., oxides, hydroxides, aluminosilicates).

In terms of aluminium determination in soil, several forms can be distinguished:

- exchangeable Al
- total Al
- extractable Al

2.9.1 Determination of exchangeable aluminium

Exchangeable aluminium is strongly bound and is active at low pH levels (pH < 5) or high pH levels if a substance that transforms ions into chelates is present. Aluminium can be extracted from soil by means of strong acids (the most common is HCl).

Laboratory equipment and reagents: analytical balance, volumetric flask (100 ml), laboratory spoon, glass tube (diameter 1.5 – 2 cm) ended with a pipe (diameter 3 mm), powdered cellulose, glass wool, 1 mol/dm^3 KCl, and distilled water.

Work process:

Fine-grained soil sample I is weighed (10 g) and placed in a glass tube ended with a pipe. Glass wool is placed in the bottom and a volumetric flask is situated under the pipe. The soil is washed with 100 ml of 1 mol/dm^3 KCl added in small doses but not longer than 2 hours. If the solution does not go through in the allotted time, the soil will be mixed with cellulose in a ratio of 1:1 and washed again. Then, the solution is diluted to the scale line with distilled water and the exchangeable aluminium is determined via titration, colorimetric methods or AAS.

2.9.2 Determination of total aluminium by means of bicarbonate fusion

The total aluminium indicates soil conditions in cases where such factors as soil-forming substrate and weathering are concerned. The total aluminium can be further used for clay mineral identification. The principle of determination lies in Na_2CO_3 fusion and consequent dissolution in concentrated HCl.

Laboratory equipment and reagents: analytical balance, platinum pot with lid (30 ml), glass rods, laboratory spoon, hair-pencil, triangle, burner, nickel pincers, beakers (50 ml, 100 ml and 400 ml), watch-glass, water bath, $HClO_4$ (70 %), concentrated HCl, Na_2CO_3 anhydride, and distilled water.

Work process:

In a platinum pot, 4 g of Na_2CO_3 and 1 g of soil are mixed together. The remaining soil is removed with a hair-pencil. One gram of Na_2CO_3 is added to the pot so that the ratio between the sample and flux equals 1:5. The pot is placed on a triangle and partially covered by a lid such that 1/10 remains open to prevent reduction. The pot is first heated opposite the lid. After 5 – 10 minutes, when the bottom has a dark red colour, the temperature rises and after another 5 – 10 minutes the content of the pot begins to fuse. It is important to ensure

that the opening of the pot is not covered by the flame. After the bubbles stop escaping, the process of fusion is complete, and the open pot is warmed for an additional 1 – 2 minutes.

Distilled water is poured onto the fuse after cooling, which is then carefully heated until the boiling point is reached. At that moment, the fuse is released and can be transferred into a 400 ml beaker. The fuse is further grained by a glass rod in 50 ml of hot distilled water. Then, 10 ml of concentrated HCl and 10 ml of $HClO_4$ are added. Finally, the beaker is covered by a watch-glass and the content is melted over a water bath. The aluminium is determined by means of AAS or colorimetric methods.

2.9.3 Determination of extractable aluminium

Extractable aluminium can be determined by ammonium acetate (pH 4.8) extraction. This method can be used for soil quality and weathering determination as well as exchangeable Al. The main principle of the determination is Al conversion into solution by means of ammonium acetate.

Laboratory equipment and reagents: analytical balance, Bűchner funnel, beakers (2 litres and 100 ml), laboratory spoon, volumetric flasks (100 ml and 1,000 ml), pH meter, 1 mol/dm^3 acetic acid, 1 M ammonium acetate, ammonium hydroxide, and distilled water.

Work process:

First, ammonium acetate must be prepared. To 400 ml of distilled water is added 58 ml of frozen acetic acid and 70 ml of concentrated NH_4OH, which is diluted to 1,000 ml using distilled water. Then, the solution is transferred into a 2 l beaker and the pH value is adjusted to 4.8 using 1 mol/dm^3 acetic acid.

Fine-grained soil sample I is then weighed (10 g) into a 100 ml beaker, to which 50 ml of 1 mol/dm^3 CH_3COONH_4 is added. The solution is stirred and allowed to stand for 2 hours, after which it is filtered in a Bűchner funnel and wash 5 times with 10 ml of ammonium acetate solution. The filtrate is stirred in a volumetric flask and filled to the scale line with distilled water. Extractable aluminium is determined by means of AAS or colorimetric methods.

2.9.4 Aluminium speciation via HPLC method

The principle of this method lies in the separation of individual Al forms on an ionic column. The identification is followed by post-column derivatisation and consequent spectroscopic detection.

Laboratory equipment and reagents: analytical balance, shaker, liquid chromatograph with ionic column, centrifuge, disc filter (pore size 40 μm), small bottles (special for liquid chromatograph), laboratory spoon, volumetric flasks (100 ml and 1,000 ml), PE bottle (100 ml), automatic pipette, 0.5 mol/dm^3 KCl, 1 mmol/dm^3 Na_2SO_4 in 7.5 mmol/dm^3 H_2SO_4 (mobile phase), concentrated H_2SO_4, 0.1 % solution of Triton in 1 mol/dm^3 NH_4Ac (derivatisation solution), standard solution of Al, and deionised water.

Work process:

All necessary reagents must be prepared in advance:

- 0.5 mol/dm^3 KCl: 37.27 g of KCl is dissolved in deionised water, the pH is regulated to 5.8 and the solution is diluted to 1,000 ml with deionised water.
- 1 mmol/dm^3 Na_2SO_4: 20 g of Na_2SO_4 is dissolved in deionised water, 0.4 ml of concentrated H_2SO_4 is added and the solution is diluted to 1,000 ml with deionised water.
- Triton: 77.08 g of NH_4Ac is dissolved in deionised water, 1 ml of a saturated solution of Triton is added and the solution is diluted to 1,000 ml with deionised water.
- Al standards: dilutions are prepared using an automatic pipette, 100 ml volumetric flasks and 0.5 mol/dm^3 KCl. It is necessary to work with at least 4 standard solutions (concentration range of 0.5 – 20 ppm).

Once the reagents are prepared, 5 g of fine-grained soil sample I is weighed into a PE bottle, to which 50 ml of 0.5 mol/dm^3 KCl is added and shaken for 24 hours. Then, the suspension is centrifuged (12,000 turns/minute) for 30 minutes and filtered into small dry bottles after the elapsed time. Determination of Al content is then performed by means of HPLC with an ionic column (λ = 310 nm).

2.10 Heavy metals in soil

Heavy metals can be characterised as elements whose specific weight is greater than 5 g/cm^3. Their origin can be natural, conditional on parent rock composition, or anthropogenic. Most heavy metals are present in the form of cations in soil and their behaviour depends mostly on soil reaction, sorption capacity and microbial activity.

Soil metals can be divided into several categories in which equilibrium is constantly being established:

- water-soluble
- exchangeable
- bound on Fe and Mn oxides
- bound on defined compounds (carbonates, sulphides etc.)
- bound on silicate structures (so-called residual fraction)

Different extraction reagents, which allow soil metals to be released, can be used in each of these categories. In general, deionised water is used for water-soluble fraction extraction. This fraction is usually determined together with the exchangeable fraction where solutions of neutral salts are used (in particular, potassium, sodium and calcium chlorides and nitrates).

Leaching reagents of organically bound metals are chelators or alkaline, and hydrogen peroxide followed by concentrated acid can be used as well.

Heavy metals bound on Fe or Mn oxides can be determined by means of hydroxylamine-hydrochloride in a leachate. However, extraction via hot oxalic acid can also be applied.

Metals bound on carbonates can be extracted by 1 mol/dm^3 sodium acetate solution, whose pH is adjusted to a value of 5 using acetic acid. Strong acids (e.g., HNO_3) are used for binding onto phosphates and sulphides.

The residual (silicate) fraction is evaluated as the difference between total content and the sum of other fractions.

2.10.1 Extraction of heavy metals from soil via aqua-regia

Aqua-regia oxidises soil samples and provides complete dissociation of sulphides and partial dissociation of the silicate matrix. The extent of solubility of individual elements

varies; the lowest is found for elements bound on resistant rock minerals (e.g., Mo, Cr, and V), whereas the highest is found for highly soluble metals (e.g., Zn and Cd).

Laboratory equipment and reagents: analytical balance, laboratory spoon, beakers (250 ml), graduated cylinder, reverse condenser, absorption dishes, sand bath, filter funnels and papers, volumetric flasks (100 ml and 1 000 ml), concentrated HCl, concentrated HNO_3 (65 %), 0.5 mol/dm^3 HNO_3, and distilled water.

Work process:

For the preparation of 0.5 mol/dm^3 HNO_3, 35 ml of concentrated HNO_3 was diluted to 1,000 ml with distilled water.

Next, 3 g of fine-grained soil sample II was weighed into a beaker to which a small amount of distilled water, 21 ml of HCl and 7 ml of concentrated HNO_3 were added. Then, 15 ml of 0.5 mol/dm^3 HNO_3 is placed into an absorption dish and connect to the reaction beaker with a reverse condenser. The solution is allowed to stand for 16 hours before heating it until a temperature of 130 °C is reached, which should be maintained for 2 hours. The condensing zone should be 1/3 of the condenser at most. After cooling, the solution is transferred to a reaction beaker, and the dish as well as the condenser are flushed with 0.5 mol/dm^3 HNO_3. The mixture is filtered into a volumetric flask (100 ml) and then diluted with distilled water up to the scale line. The final solution is measured by means of AAS.

2.10.2 Extraction of heavy metals from soil via nitric acid solution

A solution of 2 mol/dm^3 HNO_3 is commonly used to extract heavy metals from soil in which the soil to extractant ratio is 1:10. The principle of this determination lies in the soil sample oxidation, which is related to the breakdown of sulphides. The amount of elements is variable depending to the soil type and character of the individual elements.

Laboratory equipment and reagents: analytical balance, shaker, laboratory spoon, PE bottles (100 ml), pipette (50 ml), centrifuge with cuvettes, 65 % HNO_3, 2 mol/dm^3 HNO_3, and distilled water.

Work process:

To prepare 2 mol/dm^3 HNO_3, 139.5 ml of 65 % HNO_3 is diluted to 1,000 ml with distilled water. Then, 5 g of fine-grained soil sample I and 50 ml of 2 mol/dm^3 HNO_3 are placed in PE bottles, which are shaken for 6 hours. The suspensions are then centrifuged (15,000 turns/minute) for 30 minutes. The presence of heavy metals is determined via AAS.

2.10.3 Extraction of mobile forms via 0.05 M ethylenediamine-tetraacetic acid solution

Ethylenediaminetetraacetic acid (EDTA) is capable of releasing water-soluble and exchangeable metals as well as metals bound on carbonates and organic matter. EDTA links releasable forms into complexes and then transfers them into solutions.

Laboratory equipment and reagents: analytical balance, PE bottles (100 ml), shaker, pipette (50 ml), filter funnel and paper, 0.05 mol/dm^3 EDTA (pH = 7), and distilled water.

Work process:

In PE bottles, 5 g of soil sample and 50 ml of 0.05 mol/dm^3 EDTA are added and allowed to shake for 1 hour. After the elapsed time, the mixture is filtered through folded filter paper and the resulting filtrate is then analysed via AAS. Centrifugation can also be used instead of filtration.

3 References

Buckman, H. O. & Brady, N. C. (1969): The Nature and Properties of Soils. Macmillan, London, 653 p.

Buol, S. W., Southard, R. J., Graham, R. C. & McDaniel, P. A. (2011): Soil Genesis and Classification. Wiley-Blackwell, Chichester, 543 p. ISBN: 978-0-8138-0769-0.

Cassagrande, A. (1934): Die Aräometer – Methode zur Bestimmung der Kornverteilung von Boden und andere Materialien. Julius Springer, Berlin, 56 p.

ČSN 720103 (2009): Basic analysis of silicates – Determination of loss on ignition. Úřad pro technickou normalizaci, metrologii a státní zkušebnictví, 4p.

DS/ISO 11464 (2006): Soil quality – Pretreatment of samples for physic-chemical analysis. The International Organization for Standardization.

Food and Agriculture Organization of the United Nations (FAO) (2006): Guidelines for Soil Description. FAO, Rome, 97 p. ISBN: 92-5-105521-1.

Hazelton, P. & Murphy, B. (2012): Interpreting soil results: What do all the numbers mean? CSIRO, Collingwood. 152 p. ISBN: 978-0-64309-225-9.

ISO 11465 (1993): Soil quality – Determination of dry matter and water content on a mass basis – Gravimetric method. The International Organization for Standardization, 3p.

ISO 10390 (2005): Soil quality – Determination of pH. The International Organization for Standardization, 7 p.

ISO 25177 (2008): Soil quality – Field soil description. The International Organization for Standardization, 35 p.

Jandák, J., Pokorný, E., Hybler, V. & Pospíšilová, L. (2003): Cvičení z půdoznalství. Mendelova zemědělská a lesnická univerzita, Brno. 92 p. ISBN: 80-7157-733-2.

Sarkar, D. & Haldar, A. (2005): Physical and Chemical Methods in Soil Analysis. New Age International Publishers, Delhi, 193 p. ISBN: 978-81-224-2411-9.

Schachtschabel, P., Blume, H. P., Brümmer, G., Hartge, K. H. & Schwertmann (1992): Lehrbuch der Bodenkunde. Ferdinand Enke Verlag, Stuttgart, 491 p. ISBN: 3-432-84773-4.

Sposito G. (2004): The surface chemistry of natural particles. Oxford University Press, Oxford, New York, 242 p. ISBN: 0-19-511780-8.

Sposito G. (2008): The chemistry of soils. Oxford University Press, New York, 329 p. ISBN: 978-0-19-531369-7.

Valla, M., Kozák, J., Němeček, J., Matula, S., Borůvka, L. & Drábek, O. (2008): Pedologické praktikum. Česká zemědělská univerzita, FAPPZ, Praha. 151 p. ISBN: 978-80-213-0914-2.

Váša, J. (1959): Stanovení půdních hydrokonstant. Vodní hospodářství 3, Praha.

U.S. Salinity Laboratory Staff (1954): Methods of Soil Characterization. In Diagnosis and Improvement of Saline and Alkali Soils. Agr. Handbook 60, USDA Washington, D. C., pp. 83-147.

Internet sources:

http://www.echo2.epfl.ch (viewed on 6. 8. 2012)

http://www.verkon.cz (viewed on 6. 8. 2012)

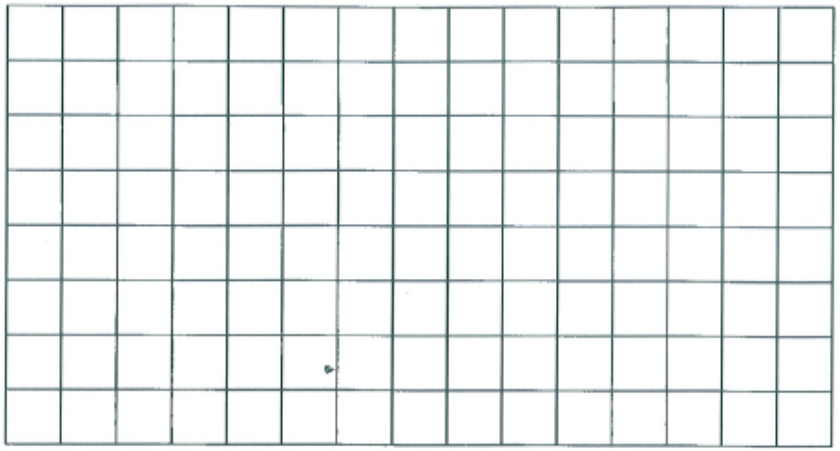
http://www.helago-cz.cz (viewed on 6. 8. 2012)

http://www.ekotechnika.cz (viewed on 10. 8. 2012)

4 Appendix

Appendix I: Field record

Scheme of the terrain cross-section outline: Altitude: _____ m.n.m.



Brief agronomic characterization of soil: _____

_____

_____

Arrangement proposal: _____

_____

_____

Soil expert: _____ Examiner: _____

Department: _____

Faculty of the Environment UJEP

Králova Výšina 7, 400 96 Ústí nad Labem

FIELD RECORD

Probe No. _____ Section: _____

Date: _____ Place: _____

Coursing No.: _____ Place name: _____

Location (meso-microrelief): _____

_____

_____

Vegetation cover and its condition (weeds): _____

_____

_____

Carbonates (bubbling): _____

Underground water: _____

Parent rock (eroded particles deposition): _____

_____

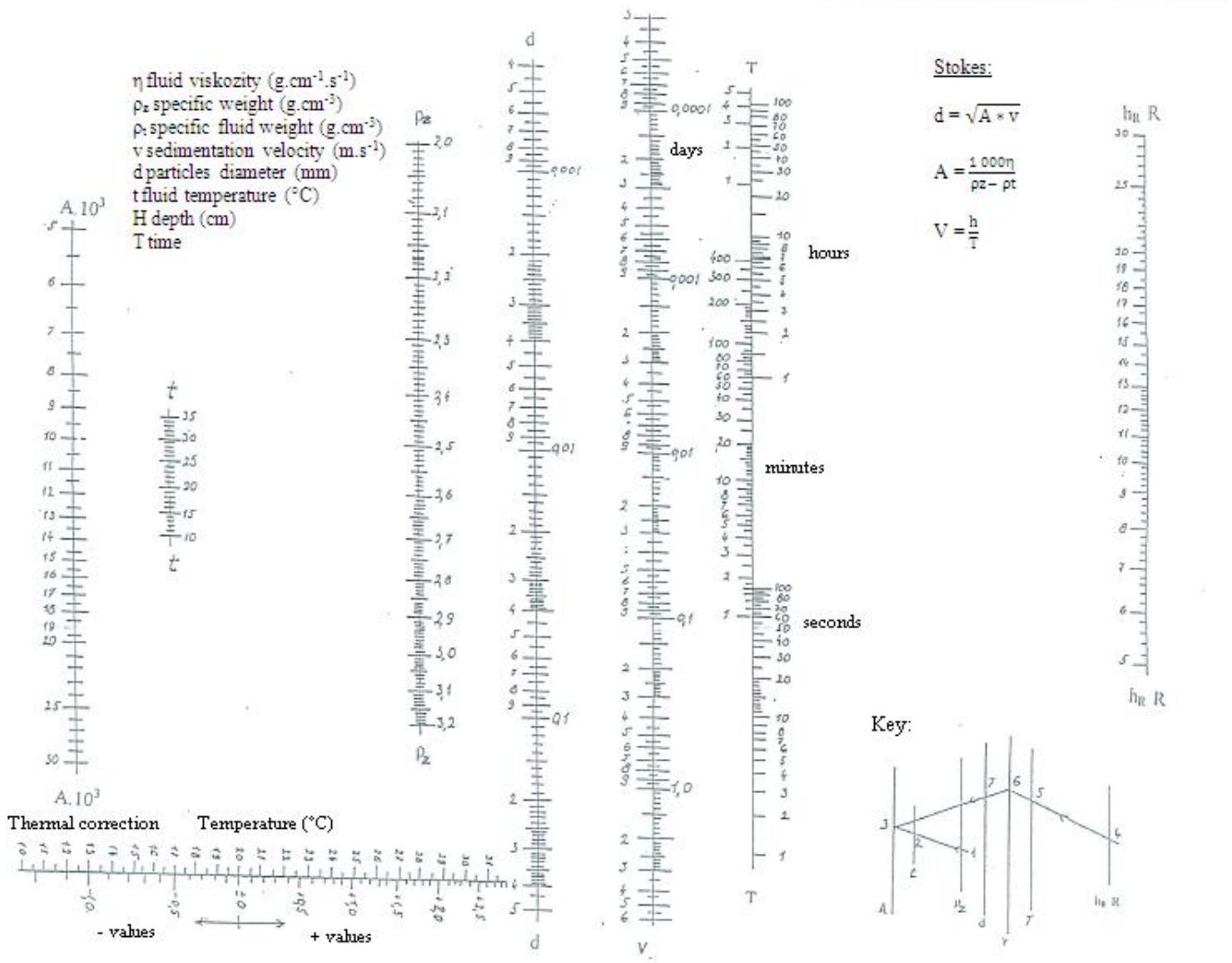
Performed soil amelioration: _____

_____

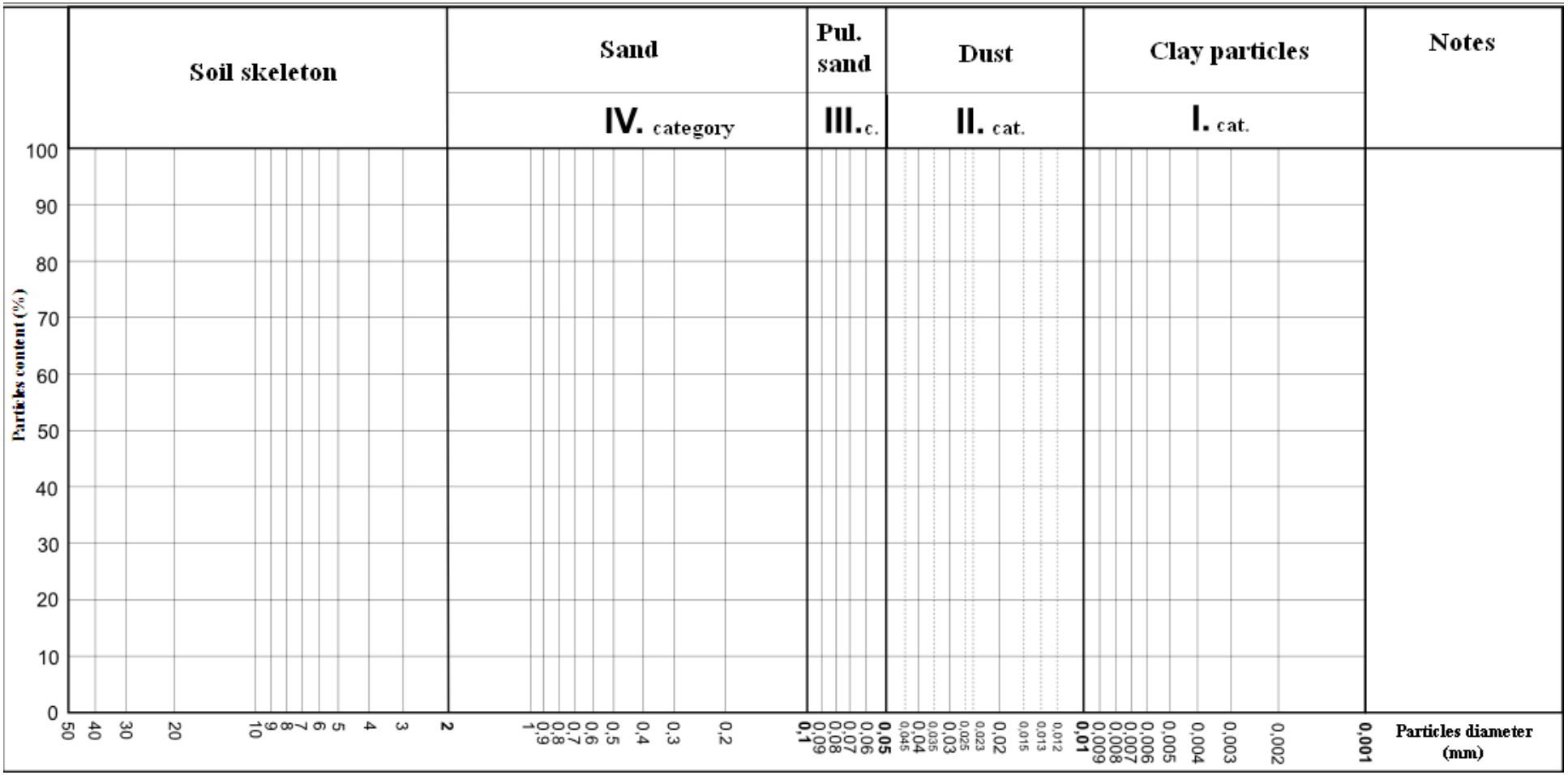
Soil nomenclature: _____

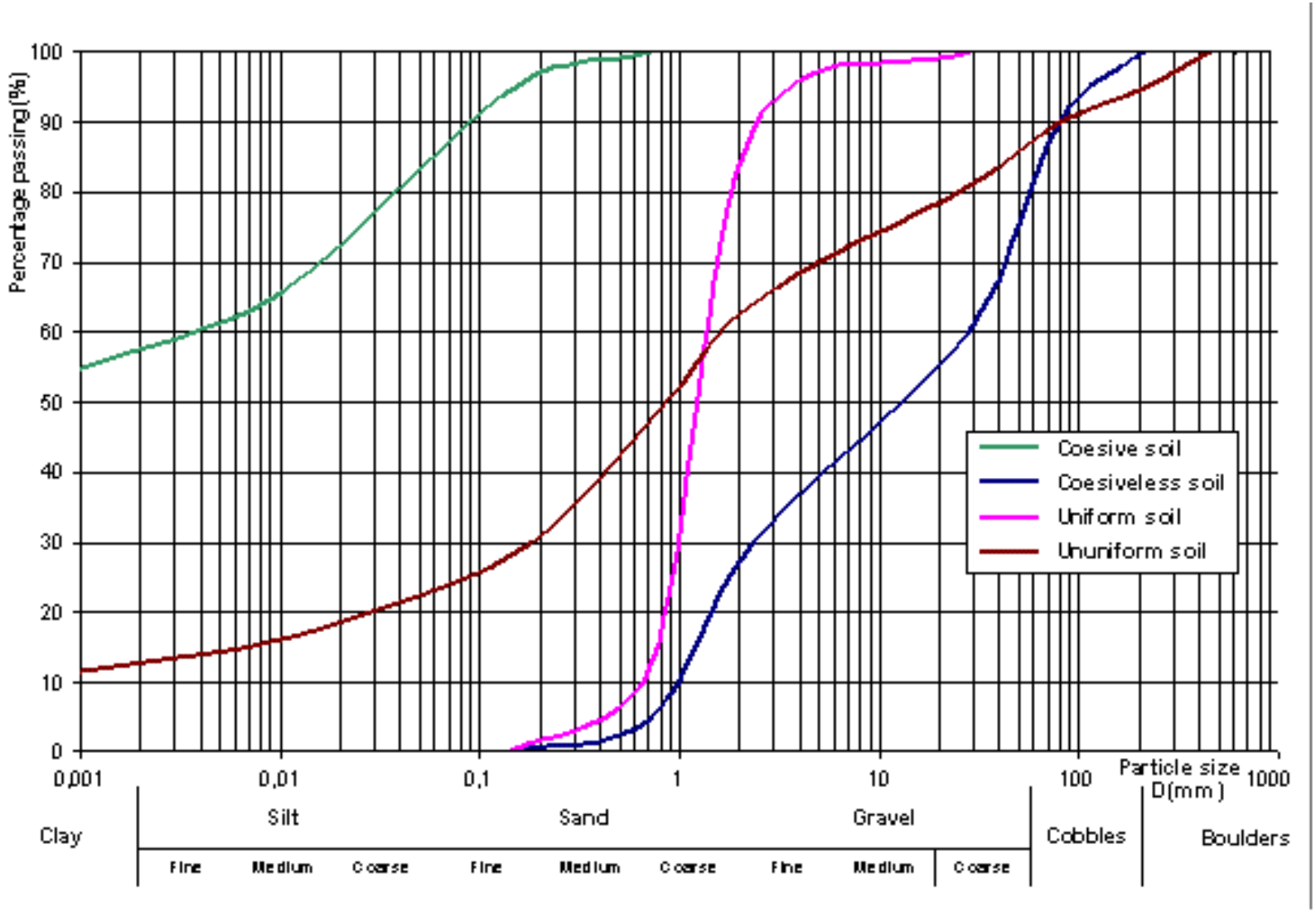
Soil profile outline	Index of genetic horizon	Morphological description of soil horizons						Samples /depth (cm)
		colour	structure	soil type	soil skeleton	moisture consistence	new formations	
	0 20 40 60 80 100 120 140 160							

Appendix II: Nomogram



Appendix III: Particle size curve





(http://www.echo2.epfl.ch)